The relationship between adverse childhood experiences and pro-inflammatory analytes and the mediating role of cortisol

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Abstract

Adverse childhood experiences (ACEs) are harmful experiences that have occurred during the developing years of life. ACEs often include maltreatment, household dysfunctions and other traumatic events. People with ACEs have been found to be at greater risk of pulmonary, cardiovascular and auto-immune diseases. Recent research has suggested that the epigenetic regulation occurring as a result of these ACEs can program macrophages to sustain inflammatory processes and therefore contribute to the development of these diseases. As one of the primary responders to stress, cortisol is also a suppressor of inflammation. Therefore, dysregulation of the cortisol levels, chronically high or low, also brought forth by ACEs exposure can affect inflammation. The purpose of the current study was to investigate the relationship between ACEs exposure and physiological measures including cortisol and different pro-inflammatory analytes. This cross-sectional study included follow-up data from 156 participants as part of the Niagara Longitudinal Heart Study. Out of the 156 participants, a final sample of 101, with 23 males and 78 females, complete with physiological measures was included in the analyses. The current study collected ACEs data from questionnaire, cortisol from hair, and inflammatory analytes including CRP, IL-6Rα, gp130, sTNFr1, sTNFr2, IFN-γ, and IL-10 from blood. Total ACEs score was negatively associated with cortisol levels. Every additional exposure to a type of ACEs decreased cortisol levels by 21.2 (pg/mg) on average. Exposure to ACEs was positively associated with IL-6Rα but was not associated with all other inflammatory analytes. Every additional exposure to a type of ACEs increased IL-6Rα levels by 284.6 (pg/mL) on average. In contrast to previous literature, sex differences from the regression analyses were also found in the current study among the inflammatory analytes CRP, IL-6Rα, sTNFr1, and IL-

10. Cortisol did not mediate the relationship between exposure to ACEs and the different inflammatory analytes. The current study was limited in properly detecting associations as the pilot sample was underpowered. The proportion of cortisol availability in males was much lower than in females. The current study found that ACEs were associated with lowered chronic cortisol and elevated IL-6Rα.

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Abbreviations

ACE – Adverse childhood experience

HPA – Hypothalamic-pituitary-adrenal

NOD – Nucleotide-binding oligomerization domain

PBMCs – Peripheral blood mononuclear cells

NF-κB – Nuclear factor kappa-light-chain-enhancer of activated B cell

IκB – Inhibitor of kappa B

TNFα – Tumour necrosis factor α

IL-1β – Interleukin-1 β

IL-6 – Interleukin-6

IL-10 – Interleukin-10

CRP – C-reactive protein

sTNFr1 – Soluble tumour necrosis factor receptor 1

sTNFr2 – Soluble tumour necrosis factor receptor 2

gp130 – Glycoprotein 130

IL-6Rα – Interleukin-6 receptor α

T_h1 – Type 1 T-helper cells

T_h2 – Type 2 T-helper cells

T_{reg} – regulatory T-cells

T_h – T-helper cells

OLS – Ordinary least squares

mTNFr1 – Transmembrane TNFr1

mTNFr2 – Transmembrane TNFr2

SES – Socioeconomic status

NSAID – Nonsteroidal anti-inflammatory drugs

PVN – Paraventricular nucleus

CRH – Corticotropin-releasing hormone

ACTH – Adrenocorticotropic hormone

Chapter 1 Introduction

There has been mounting evidence that exposure to traumatic events in childhood is associated with higher rates of morbidity and mortality later in life (Dong, 2004; Anda et al., 2009). Past research has shown that traumatic childhood events are related to health disparities in adulthood that includes cardiovascular, liver, musculoskeletal, and respiratory diseases, and cancer (Felitti et al., 1998). Investigators have proposed two main pathways in which early stress experienced in life, usually referred to as adverse childhood experiences (ACEs) can affect adult health – by lifestyle/behavioural proclivities and by biological embedding during developmental periods of life (Miller, Chen, & Parker, 2011). Through biological embedding, early life stress gets "programmed" into the immune cells that initiate and maintain inflammation, a process linked to chronic diseases of aging (Miller, Chen, & Parker, 2011). The biological embedding model also specifies that molecular mechanisms such as epigenetic pathways and post-translational modifications are responsible for the programming in immune cells. Consequently, psychosocial stress experienced during the developing years of a child gets programmed into the immune cells that renders them less sensitive to inhibitory signals and thus amplifying their pro-inflammatory tendencies. As a result, these immune cells foster systemic chronic inflammation that is characterized by elevated levels of pro-inflammatory biomarkers. Over the life course, dysregulation of cortisol, also brought forth by ACEs, creates a hormonal environment that is believed to

further amplify the pro-inflammatory tendencies of immune cells and contribute to a chronic pro-inflammatory state.

Research focussing on ACEs and these biological changes were examined in teenagers (ages 9-19 years) (Cicchetti & Rogosch, 2001; Miller & Chen, 2010) and middle to older adults (around 30-70 years of age) (Van der Vegt et al. 2009; Kiecolt-Glaser et al., 2011). Acquiring information on maltreated children places a responsibility on the researchers to report the information to child protective services which raises ethical issues in asking for such information. Therefore, some researchers rely on caregivers to report ACEs. However, there are also issues with these reports as caregivers may either be unaware of these events or provide false information to protect themselves or their families. A comparison study has shown considerable disagreement in maltreatment reporting between parents and their children (Chan, 2012). Studies that sample middle to older aged adults are also at risk for incomplete ACE data because older adults are more prone to long-term recall issues than younger adults (Maughan & Rutter, 1997).

ACEs have been linked to the development of chronic inflammation among adults as several reviews have shown that accumulation of maltreatment and household dysfunction are associated with elevated pro-inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor alpha (TNFα), and acute-phase proteins such as C-reactive protein (CRP) (Hostinar et al., 2015; Friedman et al., 2015). Furthermore, behavioural/lifestyle and biological pathways have recently been identified as potential mediators between ACEs and the development of chronic inflammation-related outcomes (Miller et al., 2011). Mediators are other factors or variables that transmit the

effects of an antecedent variable on to a dependent variable (MacKinnon & Fairchild, 2009). Identifying and testing mediator variables allow researchers to better understand and explain the relationships between different variables and the mechanistic processes.

Several studies have investigated mediators of the relationship between ACEs and behavioural outcomes and suggested that exposure to early life stress molds corticolimbic circuitry in the brain so that vigilance and mistrust tendencies are heightened (Miller et al., 2011). Through these heightened tendencies, immune cells were shown to have elevated pro-inflammatory reactions to microbial stimuli (Suarez et al., 2004). In addition, early life stress was also found to shape the neural circuitry that underlies self-regulation leading to impulsive behaviours that are known to contribute to chronic inflammation such as smoking and poor diet (Miller et al., 2011).

Physiological mediators were also thought to be a potential pathway in explaining how ACEs can contribute to inflammation (Miller et al., 2011). As shown in prior research, different types of maltreatment have been linked with altered hypothalamic-pituitary-adrenal (HPA) functioning identified through changes in cortisol levels (Cicchetti & Rogosch, 2001). As both the HPA axis and immune system are highly integrated systems that collaborate to provide an appropriate response to psychological stress, cortisol levels may further explain how ACEs contribute to chronic inflammation (Chiang et al., 2015). Although psychological and behavioural factors are potential pathways explaining chronic inflammation, the proposed study focuses on the biological pathway specifically investigating if cortisol represents a potential pathway through which ACEs contribute to chronic inflammation in young adults.

The current study examined a sample of young adults that were recruited among those who participated in a study examining cardiovascular health in children. The current study tested, cross-sectionally, if ACEs were associated with inflammation analytes as well as cortisol. In addition, mediation analyses were conducted to test if cortisol mediated the relationship between ACEs and inflammatory analytes. ACEs were operationalized as a cumulative variable summing the exposure to various types of child maltreatment and household dysfunction. Regression analyses were used to test the association between ACEs and inflammatory analytes and cortisol. It is predicted that higher number of ACEs will be associated with higher levels of pro-inflammatory analytes and higher levels of cortisol. Moreover, cortisol would mediate any relationship between ACEs and different pro-inflammatory analytes.

Chapter 2 Literature review

2.1 Adverse childhood experiences (ACEs)

While research has examined a broad array of stressors across the life course from non-events (Gersten et al., 1974) to chronic stress (Pearlin, 1989), one area that has been particularly salient in relation to physiological plasticity and programming during the sensitive developmental periods is that of childhood traumatic events. Since the seminal study by Felitti et al. (1998), ACEs have been defined as the collection of early-life traumatic experiences including physical, emotional, and sexual abuse and severe household dysfunction. More recently, others have included additional types of early-life adversities, for example exposure to a natural disaster, as well as socio-economic

adversity, low socio-economic status (SES), and poverty (Kuhlman et al., 2017; Friedman et al., 2015). While not an ACE per se, studies have examined the effect of low SES as an early life adversity as a social determinant of health (Miller et al. 2009). And while low SES and ACEs are both social determinants of health, having grown up in a low income or low education household as a child, with the exception of severe economic deprivation and homelessness, does not equate to the toxic environment of experiencing ACEs such as having been maltreated as a child.

As these social determinants occur during sensitive development periods when the immune system is malleable, exposure to ACEs or deprived economic environments can influence the programming of the body's stress response systems. The study by Miller et al. (2009) measured low SES by scaling it based on parental occupation during the first five years of the child's life with higher scores signifying lower occupational status and, hence, lower SES. With respect to ACEs specifically, one of the earliest approaches to operationalizing exposures was by a cumulative measure where an ACEs variable captured the frequency of exposures (Felitti et al., 1998). The study by Hostinar et al. (2015) used a similar method and captured the number of exposures of different types of ACEs including physical, emotional, sexual abuse, emotional and physical neglect, parent divorce, parent drug abuse, and parent depression and created groups of no exposure, one exposure, two exposures, three exposures, and four or more exposures. The idea behind the use of four exposures as a threshold originated from the ACEs study where Felliti et al. found a threshold effect of four ACEs that indicated a non-linear trajectory between negative health outcomes and the number of ACEs exposures.

Using the baseline data from the study being used in the current analysis, the parent-reported ACEs variable was dichotomized where participants were grouped into less than four ACEs exposures or four or more ACEs exposures (Pretty et al., 2013). In comparison to those with less than four ACEs, those with four or more were associated with higher heart rate, body-mass index, and waist circumference. One review discussed the different ways in which ACEs were operationalized in studies assessing cardiometabolic health (Friedman et al., 2015). They found that studies assessing a single exposure to one ACE may overestimate its effect on adult health outcomes as those that have experienced one type of ACEs were more likely to experience another, therefore having a cluster effect. As such, the more appropriate approach was to assess ACEs cumulatively to account for the clustering of these experiences.

However, most literature investigating links between ACEs and inflammation typically sample adult populations and rely on their long-term ability to retrospectively recall their personal childhood experiences (Hostinar et al., 2015; Kiecolt-Glaser et al., 2011; Roy, 2002; Van der Vegt et al., 2009). Relying on retrospective recall among adults for research on ACEs continues to be an ongoing issue in terms of reliability in collecting ACEs data. For example, one study examining the consistency between retrospective and prospective reporting of ACEs found that there was little overall agreement between prospective and retrospective reporting of ACEs (Naicker et al., 2017). As the time period between the occurrence and reporting of traumatic events increases, deficits in memory can occur such that more memory cues are needed to recall events, important peripheral details may be forgotten, and there can be tendencies to selectively forget

negative experiences (Maughan & Rutter, 1997). Although prospective reports from a birth cohort are likely the ideal choice of ACEs data collection, retrospective recall among young adults, as is employed in the current study, presents less risk of recall bias compared to middle-aged and older adult samples.

2.2 Inflammation

The following section briefly describes the process of inflammation, the functions of cells that drive this process, as well as mechanisms associated with each biomarker that is investigated in this study. Inflammation is a defence mechanism employed by both the innate and adaptive immune system in response to physical trauma or the invasion of pathogens (Ashley et al., 2012). Inflammation consists of several phases beginning with the body's detection of pathogens and/or damaged tissues. Once detected, messenger proteins such as pro-inflammatory cytokines are released from local immune cells. Cytokines travel through the bloodstream to reach other immune cells that can lead to downstream events which can either prolong, change, or resolve the inflammation process.

Derived from blood monocytes and residing in all tissues, macrophages are a type of antigen-presenting cells that play a central role in many stages of inflammation. Pathogens, tissue injury, or foreign particles that carry certain molecular patterns are detected by transmembrane toll-like receptors and NOD (nucleotide-binding oligomerization domain)-like receptors of macrophages. The detection of pathogens or tissue damage by specialized receptors activates cascading signal transductions in the macrophage that results in the release of downstream protein complexes that promotes

the transcription of pro-inflammatory genes and upregulates the production of pro-inflammatory cytokines. For example, these signal transductions can release the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) from inhibitor of κB (IκB) so that it may translocate to the nucleus and upregulate pro-inflammatory genes. Resident macrophages release pro-inflammatory cytokines as well as histamines, leukotrienes, and prostaglandins that promote the migration of leukocytes, such as monocytes and neutrophils, to the site of injury. Neutrophils create a cytotoxic environment by degranulating highly reactive oxygen and nitrogen species to destroy pathogens as well as surrounding tissue in addition to phagocytosis (Ashley et al., 2012). Monocytes that migrate into tissue spaces transform into macrophages and dendritic cells (Ashley et al., 2012). Dendritic cells are also antigen-presenting cells that migrate to secondary lymphoid tissues and present antigens to prime naïve T cells. Naïve T cells can then differentiate into different types of effector T-cells, regulatory T-cells (T_{reg}), and helper T-cells (T_h cells) that can extend or halt the inflammatory process (Ashley et al., 2012).

The complex migratory, differentiation, and activation process of immune cells mentioned previously are primarily orchestrated by soluble proteins known as cytokines. The literature that encompasses the biological embedding model has primarily focused on a triad of cytokines, specifically IL-1β, IL-6, and TNFα. These cytokines play a large role in promoting the inflammation process and are therefore pro-inflammatory. More specifically, these cytokines can facilitate the migration of cells to the site of affliction, signal for other cells to proliferate and differentiate, activate effector functions such as

killing and repair, change fluid balance, and initiate fever responses. These acute responses to physical trauma and foreign pathogens by the innate immune system are crucial for survival, without which, small infections or minor wounds would be fatal. However, it is critical that these events are regulated and controlled so that immune cells are not continually stimulated resulting in inflammation that becomes chronic.

2.3 Altered immune responses from ACEs

Previous literature has established that long term immune function is shaped by early life exposure to pathogens, micro-organisms, and allergens (McDade, 2005). These findings have fuelled the discussion of whether psychosocial stressors, such as ACEs, also play a role in shaping the immune system. While inflammation is mainly known to be activated by physical trauma and in response to pathogens, it has also been shown to be activated in response to acute psychosocial stress (Steptoe et al., 2007; Irwin & Cole, 2011; Kuhlman et al., 2017). Many studies that identify inflammation or pro-inflammatory response measure pro-inflammatory cytokines, which are typically secreted by a variety of cells including monocytes, macrophages, T-cells and B-cells.

2.3.1 Altered immune cell response from early life adversity

A wide range of social determinants have been linked to altered immune response. While some studies looked at ACEs, other have looked at a milder form of early childhood adversity such as low SES. One study investigated monocyte reactivity to bacterial and viral challenge between adults based on their childhood SES (Miller et al., 2009). Middle-aged adults classified as having grown up in low childhood SES families had leukocytes that showed more pronounced IL-6 production in response to the

bacterial and viral challenge compared to those classified as growing up in higher SES families. This type of immune cell challenge was also replicated in another study that assessed parent harshness by using the Risky Families Questionnaire (Miller & Chen, 2010). It was found that 15-19-year-old girls reared in harsh families that included measures of mental and emotional abuse, and household dysfunction, had leukocytes that showed increasingly pronounced IL-6 responses to a fixed stimulus over eighteen months. Although it was not a direct measure of leukocyte reactivity, a study by Carpenter et al. (2010) also showed that adults with a history of maltreatment had higher IL-6 response and higher IL-6 concentration in serum over time in response to acute psychosocial stress compared to non-maltreated adults (Carpenter et al., 2010). These studies have identified that exposure to various social determinants of health ranging from socioeconomic adversity to excessively harsh parenting to different types of ACEs such as maltreatment and household dysfunction during childhood had a pronounced effect on their immune cells that are consistent with the biological embedding model.

2.3.2 Chronic inflammation from ACEs

In addition to studies focusing on the pro-inflammatory response from leukocytes, other studies also measured inflammation through pro-inflammatory cytokines in serum. The study by Tayler et al. (2006) investigated the relationship between early life stress and socio-economic disparity and psychological function on CRP levels among middle-aged adults. This study used the data from Year 15 of the Coronary Artery Risk Development in Young Adults dataset which tracks predictors of coronary

artery disease from young adults to older adults (Taylor et al., 2006). At the year of assessment, there were 5,115 participants in which 3,671 adults with a mean age of 40 were examined. Early life stress was measured by the Risky Families Questionnaire which included questions on minor maltreatment and household dysfunction. A composite variable was formed based on this measure with higher scores indicating a riskier family environment. Parental education attainment was used as a proxy variable to measure childhood SES. It was found that adults who reported growing up with socio-economic adversity were more likely to report having a harsh family environment. Those that reported growing up in low SES were associated with elevated CRP that was mediated by body mass index and psychosocial functioning while those that reported having grown up in harsh family environments were associated with elevated CRP that was mediated by psychosocial functioning.

The link between maltreatment and inflammation was further supported by a longitudinal study that followed a birth cohort to the age of 32 years (Danese et al., 2007). These participants were part of the Dunedin Multidisciplinary Health and Development Study that investigated health and behaviour in a complete birth cohort from the general population of New Zealand. This study included measures of serum hsCRP levels, fibrinogen levels, and white blood cell count. Maltreatment data was collected eleven times throughout the study. Based on the maltreatment data, participants were categorized into no maltreatment, probable maltreatment, and definite maltreatment groups. Adults categorized in the definite maltreatment group were at a higher risk of elevated CRP in adulthood compared to those in the non-

maltreatment group. This relationship persisted after controlling for all co-occurring childhood and adult risk factors such as major depression, smoking, physical activity, diet, sex, and medication use.

Similarly, the study by Kiecolt-Glaser et al. (2011) investigated inflammation among caregivers with a history of childhood trauma and childhood adversity. This study included a community sample of 132 healthy older adults composed of 58 dementia family caregivers and 78 non-caregivers (Kiecolt-Glaser et al., 2011). Maltreatment was assessed by the Childhood Trauma Questionnaire (CTQ) while six questions representing other ACEs were also recorded. In a mixed linear model analyses that adjusted for differences in age, sex, body mass index, marital status levels, older adults with a history of physical, sexual, and emotional abuse had significantly elevated IL-6 and to a lesser extent, TNFα relative to those without a history of abuse. Childhood adversity included several measures of household dysfunction and were coded into three groups including no adversity exposure, exposure to one adversity, or exposure to multiple adversities.

These three studies provided evidence to suggest that exposure to ACEs are associated with a chronic inflammation in adulthood. Adults with a history of maltreatment, specifically in physical abuse, were also shown to have higher levels of CRP, IL-6, and TNFα. In addition to physical abuse, other social determinants of health such as having grown up in a low SES family has also been shown to increase levels of IL-6 and CRP. These findings of elevated pro-inflammatory levels remain consistent with literature showing evidence of elevated pro-inflammatory response to microbial challenge and acute psychological stress from leukocytes. One of the main objectives of

this study is to investigate the association between ACEs and key pro-inflammatory proteins of inflammation such as CRP, IL-6 receptor α sub-unit (IL-6Rα), glycoprotein 130 (gp130), soluble tumour necrosis factor receptor 1 (sTNFr1), and soluble tumour necrosis factor receptor 2 (sTNFr2), interferon gamma (IFN-γ), and one anti-inflammatory protein in interleukin-10 (IL-10) as shown in pathway [1] (Figure 1.). Based on the literature review, higher number of exposures to different types of ACEs is hypothesized to be associated with higher levels of pro-inflammatory and anti-inflammatory biomarkers, and acute-phase proteins.

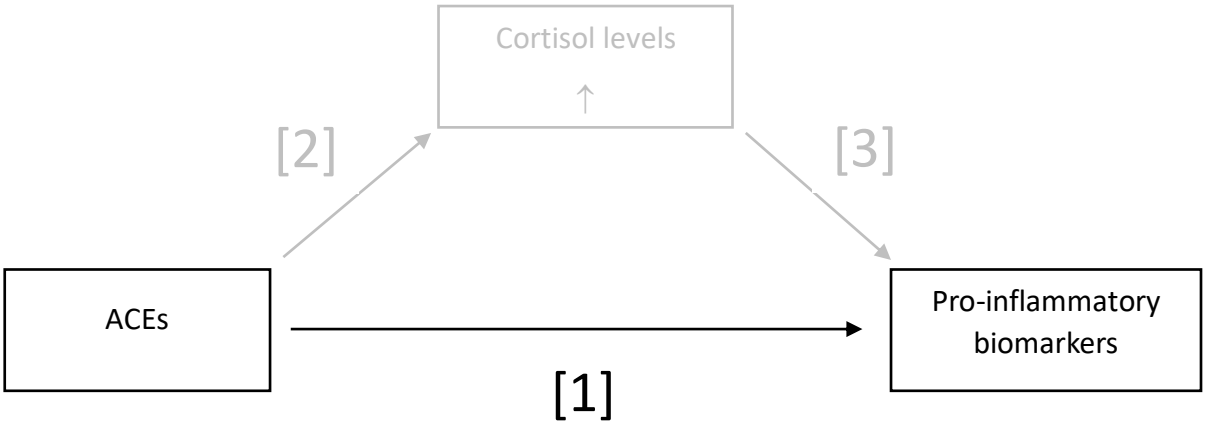

Figure 2.1 Conceptual model of study objectives pathway [1]

2.4 Biological embedding

To ground the overall concept of biological embedding, this section provides a brief primer on molecular processes that explains how the programming of immune cells occur mechanistically during developmental periods when cells have maximal plasticity to environmental outputs.

2.4.1 Molecular mechanisms of biological embedding

Epigenetics is a branch of molecular biology that studies the changes in an organism that are attributed to gene regulation. Epigenetic mechanisms such as epigenetic alterations and post-translational modifications of proteins are the primary pathways in which embedding can occur (Miller et al., 2011). Epigenetic alterations refer to altering the transcription of a certain gene in the deoxyribonucleic acid (DNA) without changing the DNA sequence. Epigenetic alterations that occur across multiple and in different locations of the gene allows for cells in the human body to have a diverse phenotype, meaning that cells are distinguishable based on their observable characteristics. This can occur through epigenetic pathways such as DNA methylation, where enzymes facilitate the binding of methyl groups to promoters, a region of the DNA that initiates transcription, controlling whether a certain gene is turned on or off. The binding of methyl groups to the promoter prevents other regulatory molecules from binding to the promoter, therefore reducing the expression of that particular gene. Another way epigenetic alterations can occur is by chromatin remodelling (Whitelaw, & Garrick, 2006). The chromatin is a structure within the nucleus of a cell that contains the DNA and proteins called histones. Chemicals can attach to histone proteins on the chromatin complex and alter the physical structure of the chromatin. Sections of the chromatin can loosen and become euchromatin, which exposes certain regions in the genome to the transcription process. In contrast, tightly coiled and packed chromatin which are genetically inactive are termed heterochromatin. Important molecules found in the biological embedding model are also susceptible to post-translational

modifications. Modification occurs when specific molecules attach to amino acids on the protein to change their structure. Structural changes are often accompanied by functional changes that can affect the activity or binding capacity of the proteins that includes the ability to pass through membranes, degradation speed, and ability to recruit other molecules. These mechanisms are important as they regulate the production of several molecular structures that, in turn, mediate the inflammation process. For example, the glucocorticoid receptor is the receptor for the stress hormone cortisol, and is found in virtually all cells. Epigenetic regulations that suppress the transcription of the gene for this receptor could result in a reduced glucocorticoid receptor availability. A reduction in glucocorticoid receptor availability that leads to a reduction in glucocorticoid receptor activity in the hypothalamus can alter the ability of central nervous system to properly detect levels of cortisol, thus allowing cortisol to escape the negative feedback loop (Raison & Miller, 2003). As a result, the typical inhibitory mechanisms of the HPA axis will not be engaged and cortisol levels will be elevated, resulting in cortisol dysregulation. The role of cortisol in relation to ACEs are described in greater detail in later sections. Similarly, upstream nucleotides that can bind to transcription factors and regulate other genes also known as response elements are also subject to modifications. As shown in the study by Miller et al. (2009), genes with response elements for the NF-κB can be upregulated through epigenetic mechanisms to increase the activity of NF-κB which leads to increased transcription of pro-inflammatory genes and consequently increased production of pro-inflammatory cytokines. In addition to the inflammation process, NF-κB is also an important transcription factor in many

developmental processes. Combining the results from animal studies that knock out the genes of certain NF-κB related machinery in animal models, the importance of NF-κB in development has been identified in this review (Hayden & Ghosh, 2004). It was shown that mice with p65/TNFR1 double knockouts hindered the initiation of innate immune responses and led to increased susceptibility to bacterial infections. IKK$\alpha^{-/-}$ mice had premature deaths due to defective epidermal and skeletal development. IKKα/β knockout mice experienced failure of neurulation during embryonic development and did not respond to TNFα, interleukin-1 (IL-1) or lipopolysaccharides. These findings suggest that the inflammatory and developmental processes share a connection through NF-κB machinery in signal transduction.

2.5 The HPA axis

The following section reviews the major endocrine system that influences cortisol levels in response to psychological stress. Bodily components that play a major role in the biological stress response identified in the biological embedding model collectively known as the HPA axis include the paraventricular nucleus (PVN) of the hypothalamus, anterior lobe of the pituitary gland, and the adrenal gland (Smith & Vale, 2006). The HPA axis is a core stress responder that operates at prolonged durations relative to other stress response systems such as the autonomic nervous system which are known to be more acute. The process of stress response in the HPA begins when stress exposure causes the synthesis and secretion of corticotropin-releasing hormone (CRH) from the PVN of the hypothalamus. CRH travels along the hypophysial portal vessels that lead to the anterior lobe of the pituitary gland and bind to pituitary corticotropes that induces

the release of adrenocorticotropic hormone (ACTH) into the circulation. ACTH primarily targets the zona fasciculata of the adrenal cortex to stimulate synthesis and release of glucocorticoids such as cortisol. Circulating glucocorticoids regulate metabolic, cardiovascular, immune, and behavioural processes by binding to glucocorticoid receptors found in virtually all cells within the human body. The HPA axis also has a negative feedback system in place to regulate cortisol levels throughout the day. Once threshold levels of cortisol are detected by the pituitary gland and the hypothalamus, the pituitary gland and hypothalamus inhibit the production of CRH and ACTH so that cortisol levels return to normal. In addition to the HPA axis, other neural systems also feedback into the HPA axis (Dedovic et al., 2009). For example, limbic systems such as the hippocampus deactivate in response to stress and negatively feeds into cortisol response. The amgydala, also an important part of the limbic system, is known for processing psychological and physical stimuli and activation of the amygdala is linked to increases in ACTH and positively feeds into cortisol response. Dysregulation of the HPA axis can lead to hypercortisolism where cortisol levels are chronically at a high level. Hypersecretion of CRH by the hypothalamus or ACTH by the pituitary gland can increase cortisol production and secretion from the adrenal glands. Researchers have also documented low cortisol that often follows elevation of cortisol. Low cortisol has been described in people suffering from stress-related disorders such as post traumatic stress disorder (PTSD) (Yehuda et al., 1995) but also in otherwise healthy people living in conditions of chronic stress, such as parents of cancer patients (Miller et al., 2002). As described in the review by Heim et al., (2000), low cortisol levels can be presented as a

result of disruption at any of the checkpoints of cortisol-related signalling in the HPA axis. These disruptions can include reduced adrenocortical secretion, reduced adrenocortical reactivity, or enhanced negative feedback of the HPA axis.

The HPA axis and the immune system are closely integrated such that the main product of the HPA axis, cortisol, in addition to other functions, serves as the main suppresser of inflammation (Webster et al., 2002). Circulating cortisol, through passive or active transport, bind to glucocorticoid receptors found within immune cells, this hormone to receptor complex translocate to the nucleus where it targets promoter or enhancer regions of the gene to modulate gene expression. One of the regions where cortisol can attach to is the glucocorticoid response element. Attachment to this region can involve the down regulation of genes that code for NF-κB, an important pro-inflammatory machinery.

Abnormal levels of cortisol are known to impact the inflammation process. During periods of development, chronic elevated cortisol can trigger the immune cells to mount a counter-regulatory response by down regulating glucocorticoid receptors to reduce binding, effectively desensitizing immune cells to inhibitory signals of cortisol and becoming resistant (Miller et al., 2002; Cohen et al., 2012; Raison & Miller, 2003). A combination of resistant cells and low cortisol levels are permissive of pro-inflammatory processes and increases the risk of inflammatory related disorders (Heim et al., 2000). This phenomenon is often shown when exposure to synthetic glucocorticoid medication induces glucocorticoid resistance (Chikanza, 2004; Corrigan & Loke, 2007). As glucocorticoid receptor activation normally functions to suppress the actions of NF-κB,

NF-κB continues to be unregulated. Unregulated NF-κB can continue to operate and promote the transcription of pro-inflammatory genes resulting in elevated levels of pro-inflammatory cytokines.

2.6 Allostasis and allostatic load

In the past two decades, the concepts of allostasis and allostatic load were brought forth by several researchers to alleviate the ambiguities of the word "stress" in physiology literature (McEwen, 2005). Previous iterations on the physiological effects of stress included Han Selye's proposed General Adaptation Syndrome, outlining the general physiological consequences that manifest from modern psychological stressors (Selye, 1950). The General Adaptation Syndrome is composed of three different stages including the alarm, resistance, and exhaustion stage. Among the three stages, the exhaustion stage was limited in describing the overall protective and damaging effects of stress mediators depending on the time course of secretion. Thus, as opposed to the exhaustion of hormones being the root of all negative consequences, it is in fact the dysregulation of hormones that can have negative consequences on the human body.

Life is maintained through homeostasis which is described as the constant equilibrium of key physiological variables such as body temperature, blood composition, and energy balance (Danese & McEwen, 2012). This equilibrium is under constant threat of disruption, as the human body is in constant interaction with the ever-changing environment and is exposed to stressors. Allostasis refers to the ability of the human body to maintain stability through change and it is through the process of allostasis that homeostasis is achieved (Danese & McEwen, 2012). The central nervous system,

endocrine system and immune system are highly integrated biological allostatic systems such that if one system undergoes change, the others are likely to be affected. For example, the activation of the immune system to defend the human body from the potential threat of foreign pathogens requires energy (Hotamisligil, 2006). Therefore, activation of the immune system would likely trigger responses of the metabolic system to block major anabolic pathways, block insulin action, and transition to catabolic pathways to provide more energy for immune processes. However, like chronic stress, exposure to trauma can constantly activate these allostatic/biological systems, whereas instead of equilibrium being maintained, equilibrium becomes disturbed, thus leading to detrimental physiological consequences (allostatic load) (Danese & McEwen, 2012). For example, in the endocrine system, exposure to ACEs has been linked to abnormally elevated cortisol levels, an indication of allostatic load (Chen et al., 2010; Cicchetti & Rogosch, 2001; van der Vegt et al., 2009; Şimşek et al., 2016; Roy, 2002). Meanwhile, in the immune system, exposure to ACEs has also been linked to increases in pro-inflammatory cytokines, also an indication of allostatic load (Taylor et al., 2006; Danese et al., 2007; Kiecolt-Glaser et al., 2011). In summary, exposure to ACEs can lead to prolonged physiological changes even after the initial threat has ceased, an indication of allostatic load. In the grand scheme, biological changes such as dysregulation of cortisol and increased levels of pro-inflammatory markers have been identified to be primary physiological pathways that link the effects of ACEs to chronic diseases of aging.

2.7 Altered HPA response from ACEs

Another objective of this study is to investigate the association between ACEs and cortisol levels. Several reviews have identified associations between ACEs and alterations to HPA axis functioning (Ehlert, 2013; Kuhlman et al., 2017; Chiang et al., 2015). Timing of exposure to ACEs were shown to influence the degree to which cortisol levels were altered: adversity exposure during late childhood (6-11) years were associated with overall greater cortisol output while adversity exposure during adolescence (13-14) were associated with overall lower cortisol output (Kuhlman et al., 2017). So far, the findings across studies examining social determinants of health including ACES and low SES are equivocal showing that exposure can either elevate or lower cortisol levels.

Among studies that present findings on elevated cortisol levels, one study investigated the longitudinal trajectories between SES and cortisol levels among children with an average age of 13 years (Chen et al., 2010). Children with lower SES parents, an indication of low childhood SES, had a higher salivary cortisol trajectory over 2 years compared to children in higher SES households. Low SES can be a source of chronic stress for some as it can bring individual effects often seen in ACEs, but its high prevalence and non-traumatic nature distinguishes itself from more traditional ACEs such as physical abuse. Moving beyond low SES as the early life adversity, Cicchetti et al. (2001) investigated different types of maltreatment in a young sample of school-aged socio-economically disadvantaged children over the span of five days. Children categorized in only the sexually abused subgroup had elevated morning salivary cortisol but were not significantly different between emotional maltreatment, physical abuse,

neglect, and non-maltreated subgroups in morning and afternoon salivary cortisol levels. In contrast, children categorized into the physical abuse subgroup had lower morning and afternoon salivary cortisol compared to children who were not maltreated. Interestingly, children categorized to both physical and sexual abuse, with three quarters of them also having experienced emotional abuse and neglect, had significantly elevated morning salivary cortisol compared to both non-maltreated children and other less diversely maltreated children (Cicchetti & Rogosch, 2001). The results showed that exposure to multiple types of ACEs is associated with elevation of cortisol in children and it also supports the hypothesis that exposure to multiple types of ACEs can lead to elevated chronic cortisol levels.

The study by Şimşek et al. (2016) specifically examined sexually abused children and found that children who were sexually abused exhibited significantly elevated serum cortisol levels compared to age- and sex-matched non-abused children. The study by Şimşek et al. (2016) also evaluated cortisol trajectory by measuring the time interval between the last date of abuse and the date that serum cortisol was measured and found an inverse relationship whereby cortisol levels decreased as the time interval increased. Even though only one type of abuse was measured, the trend of relative low cortisol levels was also observed among those with a history of severe physical abuse. Middle aged adults who were adoptees with a history of severe physical abuse had lower salivary cortisol level compared to non-abused adoptees and those with a less severe physical abuse history (Van der Vegt et al., 2009). These results align with the study by Şimşek et al. (2016), where they suggested that cortisol levels are elevated and rebound

below normal levels later in life. Yehuda and colleagues also found a similar trend in older adults. Male combat veterans suffering from post-traumatic stress disorder (PTSD) also had overall lowered 24-hour urinary cortisol levels compared to other nonpsychiatric males (Yehuda et al., 1990). They also extended these findings to another sample of older adults, this time in holocaust survivors diagnosed with PTSD (Yehuda et al., 1995).

The associations between cortisol levels and ACEs appeared to vary across age. Elevated cortisol levels were mostly found in studies examining maltreated children (Cicchetti & Rogosch, 2001) whereas abnormally lowered cortisol levels were mostly found in studies that examined adults with a history of maltreatment (van der Vegt et al., 2009).

Cortisol levels were also found to be associated with ACEs when the study included multiple types of ACEs. For instance, Cicchetti & Rogosch. (2001) reported thatthose with a history of physical, emotional sexual abuse, and neglect had either elevated or lowered cortisol. The Cicchetti & Rogosch. (2001) study did not categorize ACEs based on cumulative exposure but attempted to separate ACEs based on types. However, one of the concerns in using this method is the amount of clustering across ACEs. For example, many participants with a history of physical abuse also had a history of emotional abuse or neglect so it is difficult to disaggregate the effects of individual exposures.

Moreover, while research on children and adults exposed to abuse, neglect, other forms of early life adversity such as low SES are prevalent, the effect of other ACEs

such as household dysfunction and events such as parental death on cortisol levels have remained largely unexplored. Currently, the reviewed studies indicate an acute effect of ACEs associated with higher cortisol levels followed by a decline below normal in middle to older adults. The time at which cortisol levels start declining and at what rate has not been extensively studied. As this is one of the first studies to measure chronic cortisol among young adults, the allostatic effects of elevated cortisol levels seen during childhood report of ACEs was thought to carry forwards into young adults. Therefore, it is hypothesized that ACEs would be positively associated with cortisol levels.

2.8 The relationship between cortisol levels and inflammatory markers

Notwithstanding the links discussed above connecting ACEs to pro-inflammatory biomarkers, the dysregulation of cortisol levels as a potential mediation pathway through which ACEs predispose individuals to chronic inflammation has not been extensively studied. As cortisol normally counters the pro-inflammatory activity of monocytes, chronically low or chronic high cortisol levels can impair the control of inflammatory responses. Several studies have examined both cortisol and cytokine levels in relation to ACEs such as maltreatment and household dysfunction, and to social determinants such as low SES (Hostinar et al. 2015; Friedman et al. 2015; Miller et al., 2009). The study by Hostinar et al. (2015) examined the role of ACEs and recent life stressors in inflammation at midlife, as well as other biobehavioural mechanisms that can act as mediators such as HPA and sympathetic nervous system indices, lifestyle factors, and depressive symptoms. Participants from this study included 1,180 middle and older adults from the Midlife in the United States Biomarkers Project (Hostinar et al.

(2015). Following the study by Felliti et al. (1998), this study examined eight ACEs including maltreatment and household dysfunction to provide a cumulative score of ACE exposure. Serum levels of CRP, IL-6, fibrinogen, E-selectin, and ICAM-1 were used to provide a composite measure of inflammation, while a 12-hour overnight urinary sample (7:00 pm-7:00 am) provided a cumulative cortisol measure. An accumulation of ACEs was found to be associated with higher levels of inflammation in a multiple regression model. This relationship persisted even after controlling for other factors such as recent life events, demographic, SES, and medical history characteristics. In addition, urinary cortisol had a negative correlation with both ACEs and inflammation. Given the association between ACEs and inflammation, structural equation modeling tests were conducted to test indirect pathways that assist in explaining this association. Early findings showed that ACEs were linked to inflammation through lower urinary cortisol output. However, these findings were dropped to non-significance once adjusted for covariates as lower urinary-cortisol levels were found to be significantly associated with being African American, male, taking antihypertensive medication or cholesterol-lowering medication, corticosteroids, and having a history of diabetes.

In the study by Friedman et al. (2015), researchers investigated the associations between social determinants of health including low SES and ACEs such as physical abuse and a composite biological risk scores among middle aged adults. The composite biological risk score included measures of both HPA activity (cortisol) and inflammation (CRP, fibrinogen, IL-6 and the soluble adhesion molecule-1) as well as cardiovascular functioning (resting systolic and diastolic blood pressure, and resting pulse), sympathetic

nervous system (epinephrine and norepinephrine) , parasympathetic nervous system (heart rate variability), lipid metabolism (high-density lipoproteins cholesterol, triglycerides, body mass index, and waist-hip ratio), and glucose metabolism (glycosylated hemoglobin, fasting glucose, homeostasis model of insulin resistance). This biological risk score captured the multiple biological pathways through which stressful experiences could lead to chronic disease. This study found that an accumulation of total adversity was positively associated with a higher biological risk score (Friedman et al., 2015). Moreover, among all measures of early life adversity, low parental education and physical abuse significantly predicted a higher overall biological risk score in a single model, while others such as parental death and divorce, on welfare, and perceived low income did not. Within the overall biological risk profiles, overnight urinary cortisol levels were not significant while inflammation measures in the composite adult biological risk score that included plasma levels of CRP and IL-6 were statistically significant. Unlike the study by Hostinar et al. (2015), the study by Friedman et al. (2015) did not test for cortisol mediation.

The study by Miller et al. (2009) also explored the immune and endocrine stress response systems in relation to low childhood SES by genome-wide transcriptional profiling and biomarker measurements. This study recruited 103 healthy middle-aged adults that were categorized into low and high early-life SES that were also adjusted by their current SES (Miller et al., 2009). Inflammation was measured systemically through serum levels of CRP while the dynamics of inflammatory signalling pathways under microbial challenge were measured by quantifying IL-6 production after stimulation with

toll-like receptor ligands in serum monocytes. Transcriptional profiling of peripheral blood mononuclear cells (PBMCs) has identified relative down-regulation of genes with response elements for the glucocorticoid receptor in the low early-life SES group and significant up-regulation of genes bearing NF-κB response elements compared to the high early-life SES group. These findings of up-regulated response elements for NF-κB and down-regulated response elements for glucocorticoid receptor show that inhibitory signalling pathways in which cortisol operates through are less 'heard', which is consistent with the enabling of pro-inflammatory signalling pathways. While both low and high childhood SES groups had similar serum levels of CRP, the PBMCs of the low childhood SES group produced more IL-6 in response to simulated viral and bacterial challenges. Subjects in the low early life SES group also had greater overall cortisol output compared to those in the high early life SES group. Moreover, among those categorized in the low early life SES group, findings of down-regulated response elements for the glucocorticoid receptors and the up-regulated responses elements for the NF-κB are consistent with the higher production of IL-6 in response to microbial stimuli in an environment of greater cortisol output.

The studies by Chen et al. (2010), Ciccheti et al. (2001), Van der Vegt et al. (2009), and Miller et al. (2009) obtained cortisol measures through saliva samples, while cortisol measures by Şimşek et al. (2016) were obtained through blood serum. Measuring cortisol from saliva or serum are known to yield similar results with salivary cortisol being less invasive, however, both these methods represent a point estimate or an acute measurement of HPA activity (Meyer et al., 2014). Some studies included several

measurements throughout the day to provide a rough composite index of cortisol that are indicative of longer periods of cortisol levels. The study by Roy (2002) measured cortisol from a 24-hour urine sample while the studies by Hostinar et al. (2015) and Friedman et al. (2015) measured cortisol from overnight urine samples. Measuring cortisol through urine also provides short periods of HPA activity that spans around one day. Multiple collections of acute cortisol measures may lead to varying results. For example, the study by Ciccheti et al. (2001) collected four salivary cortisol samples every 6 months, over the span of two years, while the study by Van der Vegt et al. (2009) collected four samples of salivary cortisol in one day.

In sum, the evidence that connects cortisol with inflammation levels is inconclusive. Despite the evidence suggesting that lower SES and ACEs were associated with both greater activation of the HPA axis and elevated inflammation levels, the evidence of cortisol as a mediator in the relationship between ACEs and inflammation is scarce. Furthermore, only one study specifically examining ACEs (Hostinar et al., 2015) directly tested whether cortisol was associated with inflammation. Although the study by Miller et al. (2009) examining SES did not directly tested for cortisol mediation, the up-regulated response elements for NF-κB and down-regulated response elements for glucocorticoid receptor indicate that elevated cortisol levels found in this study had some sort of role in the increased pro-inflammatory tendencies of monocytes. Like the Hostinar et al. study, the final objective of this study was to investigate the indirect effects of ACEs on inflammation via cortisol, through pathway [2] and [3] (Figure 1.).

While chronically high or low cortisol can have an impact on inflammatory processes, the current study investigates if cortisol mediates any pro-inflammatory effects of ACEs.

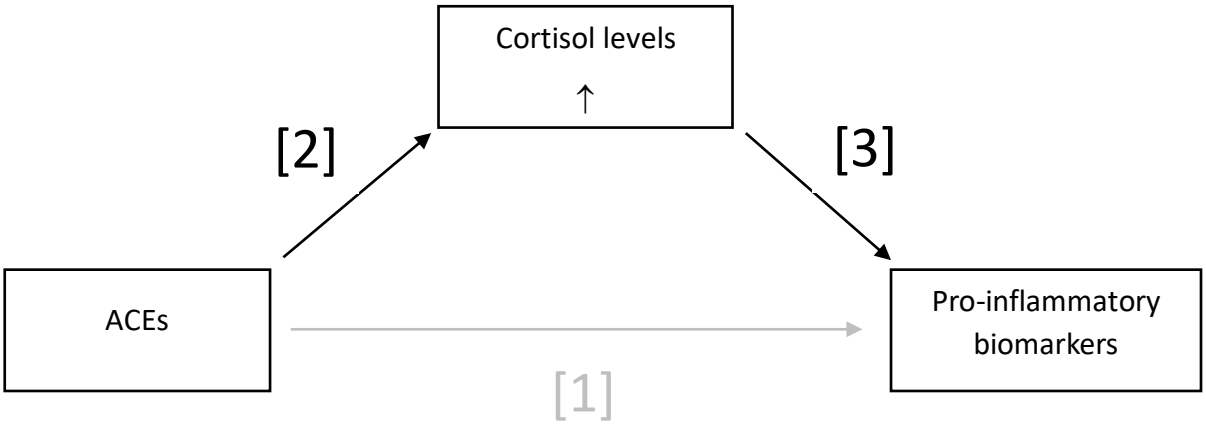

Figure 2.2 Conceptual model of study objectives pathway [2] and [3]

2.9 Summary

Studies that examined inflammation focused on socio-economic adversity (e.g., Miller et al., 2009) and a variety of ACEs including maltreatment (e.g., Danese et al., 2007) and household dysfunction (e.g., Taylor et al., 2006). Overall, adults with a childhood history of SES adversity and ACEs such as maltreatment were consistently found to exhibit higher levels of IL-6. To better understand how the HPA axis and immune system are affected by ACEs, the current study tests if there is a role in cortisol to mediate the relationship between ACEs and pro-inflammatory markers. Recent research has identified potential behavioural/psychological mediators between ACEs and inflammation. However, the examination of physiological mediators such as cortisol in research has been limited. While the study by Miller et al. (2009) did not include mediation analysis or focus on ACEs specifically, glucocorticoid resistance was found in children with low SES. Children with low SES had elevated cortisol and an elevated

immune response to toll-like receptor stimulation in PBMCs compared to high childhood SES.

Lastly, the current study also investigates how different types of maltreatment affect cortisol levels. Several studies have shown associations between and cortisol levels and SES (Chen et al., 2010) and ACEs (Cicchetti & Rogosch, 2001). However, the overall findings appear to be contradictory based on both the age of study participants and the different types of exposure. Specifically, adult combat veterans and holocaust survivors and adults reporting a childhood history of maltreatment tend to show abnormally low levels of cortisol (van der Vegt et al., 2009; Yehuda et al., 2002), whereas children exposed to socio-economic adversity and certain ACEs such as sexual abuse tend to show chronically high levels of cortisol (Cicchetti & Rogosch, 2001; Chen et al., 2010; Şimşek et al., 2016). These findings suggest that the time since exposure to adversity and the type of exposure may influence cortisol levels.

2.10 Specific aims and research questions

The current study works to investigate the associations between ACEs and relevant physiological measures as informed by the biological embedding theory.

1. The first aim of the study was to investigate if ACEs were associated with pro-inflammatory analytes including CRP, gp130, IL-6Rα, sTNFr1, sTNFr2, IFN-γ, and IL-10. While other research focuses on cytokines, the current study focuses more so on soluble receptors. Collectively, it is hypothesized that these inflammatory analytes will be associated with ACEs in positive dose-response relationship similar to the respective cytokines in previous studies.

2. The second aim of the study was to investigate if ACEs were associated with cortisol. The body of literature investigating teenagers or younger show a trend of elevated cortisol levels. Because the physiological effects of biological embedding appear to develop over time, it is hypothesized that, similar to children, young adults with higher exposure to ACEs, both total ACEs including maltreatment and household dysfunction as well as maltreatment by itself, will have elevated levels of cortisol In addition to these bivariate associations, covariates including sex, NSAIDs usage, prescription medications usage, and smoking status are also included in hierarchal models to identify other plausible explanations for any associations. As part of an exploratory analysis it was hypothesized that there would be differences in biomarkers levels between sex across both ACE exposure and cortisol levels. Therefore, tests of interactions between sex with ACEs and between sex with cortisol were included in regression analyses.

3. Finally, the third aim of the study was to investigate if cortisol mediates the relationship between ACEs and the inflammatory analytes. The ability of cortisol to mediate is difficult to hypothesize as it relies heavily on statistical associations from the first and second aim of the study. To remain consistent with the first two hypotheses, it is hypothesized that both cortisol and the inflammatory analytes were expected to be elevated in relation to ACE exposure if mediation was present.

Chapter 3 Methods

3.1 Sample

This study was part of the larger Niagara Longitudinal Heart Study (NLHS) and included a total of 68 males and 88 females. The NLHS currently investigates how ACEs amplify the risk of cardiovascular disease development and recruited participants from the earlier Heart Behavioural and Environmental Assessment Team (HBEAT) study. The HBEAT study used a systematic sampling strategy to recruit adolescents aged 11-14 years and their parents from one school board in Southern Ontario (Pretty et al., 2013). The age of males in this study ranges from 19.14 to 25.00 years with an average age of 22.25 years and the age of females in this study ranges from 19.36 to 24.47 years with an average age of 21.87 years. The initial phase of the sampling in HBEAT began in the Fall of 2007 and concluded in the winter of 2008 that led to the second phase that occurred in the spring of 2011 (Wade et al., 2019). The NLHS conducted follow up testing approximately 10 years afterwards, beginning in Spring of 2017 and ongoing. Participants were recruited for follow up testing through previous telephone contact information and by social media-based platform recruitment that used Facebook, Linkedin, Instagram and Twitter. Only participants that have made their profiles publicly accessible and have provided consent to the use of their data for future analysis from the previous study were recruited through social media. Participants that met this inclusion criteria and who were also found on social media platforms were sent a private electronic letter of introduction that identified the study that reminded them of their previous participation in the past study and asked if they would like to continue in the current follow-up study.

The NLHS has been approved by Brock University's Bioscience Research Ethics Board since January 9th, 2017 and has been renewed until the expiration date of May, 1, 2020 (file numbers 16-078-WADE and 18-288-WADE).

3.2 Data collection

3.2.1 Data collection procedure

The study testing took place at the Human Hemodynamics Laboratory at Brock University (Wade et al., 2019). If the participant was recently sick or identified any recent antibiotic use, the appointment was rescheduled. Participants were also asked to be 4 hour fasted prior to testing. Prior to testing, the participants were informed about the study protocol and informed consent was completed. Participants were also asked to provide permission to keep their data on file for future analysis. Prior to any stage during testing, participants were informed of the test procedures and how it would feel during testing as well as their right to refuse any aspect of testing without consequences. At the start, participants were asked to void their bladder and change their clothes to a tank top and shorts. A series of physiological and anthropometric measurements including resting heart rate, resting blood pressure, height, weight, waist and hip circumference were taken. Fat mass, fat-free mass, and body fat percentage was measured via air-displacement plethysmography. Participants were then placed in a supine position and fitted with equipment in preparation for a series of non-invasive cardiovascular measures that included cardiovagal baroreflex sensitivity, arterial stiffness and thickness, cardiac size and function, and cardiac function through an orthostatic-induced stressor.

After cardiovascular testing, a licenced phlebotomist performed serum collection by drawing blood through the antecubital fossa into a SST™ Serum Separation Tube (BD Biosciences, #367986). Blood was allowed to clot for 30 minutes before being centrifuged at 3000 x g for 15 minutes at 4ºC. Serum was aliquoted and stored at -80ºC. The blood serum provided all information on inflammatory biomarkers for the study. After blood collection, participants that had adequate amounts of hair provided 3 centimetres in length and a pencil width of hair taken from the back of their scalp for 3-month retrospective chronic cortisol analysis. Next, the participants were directed to spit in a saliva collection tube for DNA analysis (e.g telomere length).

After biospecimens were collected, participants were given a snack and a brief break from testing prior to completing a self-report questionnaire. Before administering the questionnaire, the research assistant ensured that only themselves and the participant were present in the room due to the sensitive nature of the questionnaire. Participants were also ensured that the questionnaire was confidential, that the information was password protected, and that a third party with no access to their personal information would enter in their data. The questionnaire was administered in a self-reported format to increase the likelihood and accuracy of reporting sensitive measures without interference. The entire data collection procedure took approximately 4 hours to complete and participants were given an $100 honorarium for their participation.

3.3 Measures

3.3.1 Chronic cortisol level

Chronic cortisol levels were measured through the scalp hair (Meyer et al., 2014). Estimating a growth rate of about 1 cm per month, the first three centimeters of harvested hair closest to the scalp was retained for analysis that provided a measure of 3-month chronic cortisol (Meyer et al., 2014). Hair collection was performed by a research associate of the NLHS. The first wave of hair samples were sent to Nipissing University for extraction and analysis. Working with the project coordinator, I conducted the cortisol extraction and quantification for the rest of the hair samples. There was no variability in cortisol data across the different labs. The process of extraction and quantification are described below. First, the hair samples were weighed to provide a quantification of cortisol per given mass at the end of extraction, washed with 100% isopropanol and left to air dry for 24 hours to remove excess cortisol from exterior sources. Second, the washed hair samples were prepared for extraction. As efficient cortisol extraction requires large surface area interaction between the hair medulla and the extraction solvent, hair samples were minced to fine pieces using surgical grade scissors in a centrifuge tube. Third, cortisol was extracted from the prepared hair samples in a three-step method to increase yield of hair cortisol concentration. Single step methods were shown to only yield 40-60% of absolute hair cortisol, while a two-step method was shown to yield 98-100% of hair cortisol (Greff et al., 2019).

To begin the first round of extraction, a 1:1 ratio of methanol was added to each hair sample to a total of 1 mL, these samples were then vortexed to completely mix the

solvent and the hair together, and were then placed in a heated plate shaker at 52°C at 100 rpm for 16 hours. Next, 0.5 mL of acetone was added, and the sample was vortexed again. Methanol and ethanol are organic solvents that are used to extract cortisol from the hair. After adding the acetone, the samples were centrifuged at 2000 rpm for 2 minutes and the solution that contained the cortisol were removed and placed into separate tubes. The cortisol-containing tubes were left open to evaporate the ethanol at RT for 24 hours. Once the liquid was evaporated, leaving only the cortisol and co-isolated residues, the first extraction step has concluded. The cortisol-containing tubes were stored in the fridge until the next extraction.

A total of three extractions following the same process as the first extraction was conducted for each of the samples. The residue from the extractions were re-suspended in 0.25 mL of phosphate buffered saline to create a solution that was used in the DRG salivary Cortisol ELISA kit (SLV-2930R) to quantify the amount of active free cortisol in the solution. The ELISA technique is based on a competitive indirect sandwich ELISA principle in which the enzyme conjugate competes with the sample cortisol to bind with the antibodies in the wells. The color change is caused by the sandwich complex that is created from the attachment of the solution substrate attached to the enzyme conjugate bound to the antibody. The assay kit contains a 96-well plate with the microtiter wells coated with anti-cortisol antibody, standards, controls, enzyme conjugate, substrate solution, and the stop and wash solutions. To begin the assay procedure, the number of microtiter wells to be used were identified and labeled on a 96-well ELISA plate template. The assay was ran in duplicate, where two wells were used per study

participant and the resulting cortisol values were averaged. Next, 100 µl of the standards from 0-6, controls low/high, and cortisol-containing samples were dispensed into the appropriate wells. All the wells were coated with monoclonal antibodies that contained the binding site for an antigenic site on the cortisol molecule. Next, 200 µl of the enzyme conjugate was dispensed into each well and mixed thoroughly through reverse pipetting. The enzyme conjugate is a horseradish conjugate that competes with the sample cortisol to bind with the antibodies. Afterwards, the plate was incubated on a plate-shaker at 300 rotations per minute for 1 hr at RT to encourage all the ligands in the solution to fully bind to the antibodies in the wells. Next, the solutions in the plate were disposed of and the microtiter wells were rinsed five times with 400 µl of diluted wash solution using a manual microtiter plate washer. After each rinse with the manual microtiter plate washer, the plates are struck on absorbent paper to remove residual droplets. Next, 200 µl of substrate solution, tetramethylbenzidine, are added to each well. This substrate attaches to the horseradish conjugates and the attachment creates a blue color change in the solution. The intensity of the color change is inversely proportional to the amount of cortisol bound top the wells. After adding the substrate, the wells are incubated for 30 minutes at RT. After 30 minutes of incubation, 100 µl of stop solution was added to each well to stop the enzymatic reaction. Within 10 minutes after adding the stop solution, the absorbance of each well was read on a microtiter plate reader at a wavelength of 450 nm.

3.3.2 Inflammatory biomarkers and cytokines

Inflammation was quantified at the protein level by measuring concentrations of key inflammatory biomarkers in the blood serum (Wade et al., 2019). Blood samples were collected by a licenced nurse/phlebotomist, allowed to clot at room temperature for 30 minutes before being centrifuged at 3000 x g for 15 minutes at 4°C, aliquoted to acquire the serum fraction using the gold-cap blood collection tube (VWR) (CABD367986L) and stored at a temperature of -80° C in single-use aliquots until they were analyzed. Serum levels of inflammatory markers were measured by using a magnetic bead-based multiplexing antibody assay (Bio-Rad CAT # 171-AL001M) and analyzed on a MagPix (Luminex) instrument. Under supervision of the study coordinator, I assisted and conducted the multiplex assay. The magnetic bead-based method uses beads that are coupled with different capture antibodies that react with the sample biomarker of interest (Figure 3.3). The attachment of the biomarker of interest on the bead's antibodies along with the biotinylated detection antibody and the detection molecules formed the final sandwich complex (Figure 3.3). The samples were read on a Luminex-based platform where the magnetic beads are illuminated to provide the classification of the biomarker and the amount of fluorescent phycoerythrin indicated the quantity of that specific biomarker attached to that specific bead.

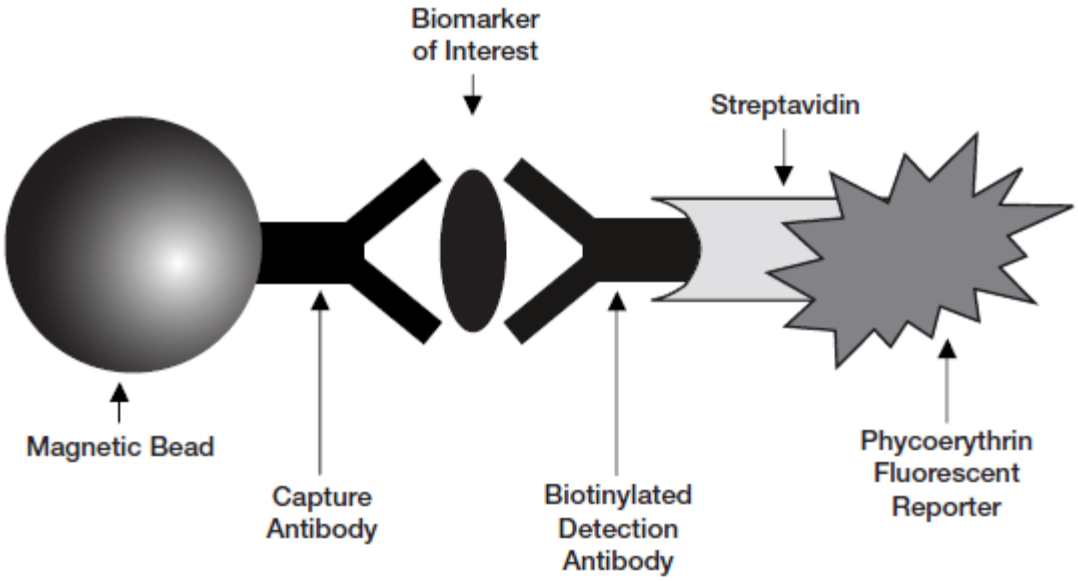

Figure 3.1 Pictorial schematic of magnetic bead-based ELISA

The assay captured 37 different inflammatory biomarkers and cytokines including pro-inflammatory receptors such as sTNFr1, sTNFr2, gp130, IL-6Rα, an acute-phase protein including CRP, and cytokines including IL-10 and IFN-γ. Prior to analysis, wash buffers were prepared, and standards and controls were reconstituted and diluted nine times according to the instructions. The 96-well ELISA plate template was used to identify and label the wells that were used. To adhere the magnetic beads to the plate 50 µl of coupled beads were added to the wells and washed twice with a manual plate washer. Wash buffer was added to the full volume of the wells and discarded. The plate was then inverted and blotted against a clean paper towel to remove excess liquid. Following the plate preparation, 50 µl of standards, samples, and controls were vortexed and added to the wells and the plate was sealed and incubated on a shaker at 850 rpm for 1 hr at room temperature. Following incubation, the plate was washed three times following the same process previously mentioned and 25 µl of the diluted antibody was

added to each of the wells and incubated at 850 rpm for 30 minutes at room temperature. Following incubation, 50 µl streptavidin-PE was added to the wells and the plate was sealed and incubated at 850 rpm for 10 minutes at room temperature. The streptavidin attaches to the biomarker of interest and the phycoerythrin serves as a fluorescent indicator. Following incubation, the plates were washed three times following the previous method. To re-suspend the beads for plate reading, 125 µl of assay buffer was added to each of the wells. Following the bead resuspension, the plate was sealed and placed on a plate shaker at 850 rpm for 30 seconds at room temperature. The quantity of analytes on the plate is then read on the Bio-Plex MAGPIX Multiplex reader.

CRP was measured using a quantitative sandwich ELISA kit by R&D Systems (DCRP00). The assay kit included the CRP microplate, enzyme conjugate, CRP standard, calibrator diluent RD5P, assay diluent RD1F, color reagent A, color reagent B, wash buffer concentrate, stop solution, plate sealers and package insert. Under supervision of the study coordinator, I assisted and conducted the CRP assay. This process proceeded in the following steps. To prepare the standard dilutions, 20 mL of the calibrator diluent RD5P was added to 80 mL of water to create a diluted control. Next, 200 µl of the calibrator diluent was added into six 25 ng/mL tubes followed by 200 µL of CRP standard into the first tube. After adding CRP standard into the first 25 ng/mL tube, the solution was mixed thoroughly and 200 µL of the solution in the first tube was added to the second 25 ng/mL tube. This process continued to the sixth 25 ng/mL tube and created a dilution series where the CRP standard (50 ng/mL) served as the highest standard and the diluted

calibrator diluent RD5P served as the control. The serum samples were thawed and allowed to clot for 30 minutes at room temperature before they were centrifuged for 15 minutes at 1000 x g. The serum samples were diluted 100-fold to prevent cross binding of the assay antibodies with the antibodies present in serum. After preparing all the reagents, the CRP microplate to be used were identified and labeled on a 96-well ELISA plate template. Next, 100 µL of assay diluent RD1F was added to each microtiter well. Next, 50 µL of standard, control, or sample were added to their respective wells. Afterwards, the plate was covered with an adhesive strip and incubated for 2 hours at room temperature. After two hours of incubation, the wells were filled with 400 µL of wash buffer using a manual plate washer and the liquid inside the wells were discarded. After discarding the liquid, the plate was blotted on absorbent paper to remove residual fluid. To fully remove the unbound reagents in the well, this wash process was repeated a total of four times. Next, 200 µL of CRP conjugate was added to each well. After adding the CRP conjugate, the plate was covered with a new adhesive strip and incubated for 2 hours at RT. During this incubation period, the CRP conjugate attaches to the sample CRP, creating a direct sandwich complex as the conjugate attaches to the sample CRP. After 2 hours of incubation, the wash process was repeated for a total of four washes. Next, the substrate solution was prepared by mixing together the provided color reagent A and color reagent B to create the substrate solution. Next, 200 µL of substrate solution was added to each well. The plate was covered from light and incubated for 30 minutes at room temperature. During this incubation period, the substrate attaches to the CRP conjugate that changed the colorless solution to blue based on the amount of

attachment between the substrate and the CRP conjugate. After 30 minutes of incubation, 50 µL of stop solution was added to each well changing the color from blue to yellow. The addition of stop solution stops the color development. Within 30 minutes since the addition of stop solution, the plate was read at an optical density of 450 nm with a wavelength correction of 540 nm.

Chronic inflammation is often measured through the quantification of pro-inflammatory cytokines. The current study, however, measured chronic inflammation through the quantification of other pro-inflammatory biomarkers that are associated with IL-6, TNFα, and macrophage activity. This study specifically focuses on analytes such as IL-6Rα, gp130, sTNFr1, sTNFr2, acute-phase protein including CRP, and cytokines including IL-10 and IFN-γ. TNFα is a key mediator in the pro-inflammatory response that exerts its biological effects through interaction with sTNFr1 and sTNFr2 (Cabal-Hierro & Lazo, 2012). After attachment to sTNFr1 and sTNFr2, this complex binds to transmembrane TNFr1 (mTNFr1) and transmembrane TNFr2 (mTNFr2) counterparts. Activation of both transmembrane receptors leads to downstream pro-inflammatory stimulus while only mTNFr1 induces cell apoptosis. Therefore, sTNFr1 and sTNFr2 are mediators of TNF-related activities. Overall, elevated TNFα, sTNFr1 and sTNFr2 were found to be associated with a host of factors that were reflective of inflammatory related conditions such as metabolic syndrome, impairments of cardiac function, and renal function (Safranow et al., 2009; Pai et al., 2004; Neirynck et al., 2015).

Similarly, IL-6Rα is the soluble receptor for IL-6. Once IL-6Rα is attached to IL-6, the IL-6/IL-6Rα complex can bind to gp130, a transmembrane protein that activates

downstream signalling pathways that can lead to the secretion of pro-inflammatory molecules (Gabay, 2006). For example, one animal study has shown that the development of atherosclerosis was stunted by inhibiting IL-6 signalling complexes, specifically gp130 (Luchtefeld et al., 2007). In the study by Luchtefeld et al. (2007), gene knockout mouse for gp130 in an atherosclerotic prone environment were less prone to developing aortic atherosclerosis, this was characterized by the decrease of macrophages in lesions.

CRP is a positive acute phase protein that increases up to 1,000-fold and is released by the liver in response to pro-inflammatory cytokines like IL-6 via activation of gp130 (Luchtefeld et al., 2007; Pai et al., 2004). CRP can activate the complement cascade, an important non-specific inflammatory defence, as well as mediate phagocytosis, regulate inflammatory responses and it is often used as a biomarker for predicting risk of coronary heart disease (Luchtefeld et al., 2007; Sproston & Ashworth, 2018). The pro-inflammatory responses are quickly followed up with anti-inflammatory responses to limit the damage to host tissue.

The cytokine IL-10 is a potent anti-inflammatory cytokine that is essential to the overall process of inflammation, specifically in inflammation resolution and shifting the process to a more adaptive immune response (Sabat et al., 2010). This cytokine is produced by many immune cells but mainly in Type 2 T-helper (T_h2) cells and T_{reg} cells. In monocytes and macrophages, IL-10 induces transcriptional activity that inhibits important pro-inflammatory functions such as antigen presentation and cytokine release (Sabat et al., 2010).

IFN-γ, another cytokine of interest in this study, is mainly produced by Type 1 T-helper (T_h1) cells that can target macrophages to promote cell-mediated immunity and phagocyte-dependent inflammation (Romagnani, 2000). Macrophages have different types of inflammatory responses, one of which is the M1 response. In response to IFN-γ, macrophages are skewed to an M1 response which are often characterized by enhanced antigen presentation to T-cells and pro-inflammatory cytokine secretion such as IL-6 and TNFα (Schroder et al., 2004). The collection of analytes listed above that includes four soluble receptors, and one acute-phase protein, and two cytokines have been associated with a wide range of diseases involving chronic inflammation.

3.3.3 Adverse Childhood Experiences (ACEs)

The Childhood Trust Events Scale (CTES) is a 26-item questionnaire that was used in this study to measure ACEs such as maltreatment, and household dysfunction consistent with research by Fellitti et al. (1998) and other types of traumatizing events such as being bullied, separated from parents and family, and having lived in impoverished conditions. Participants answered either “Yes” or “No” to these experiences that have occurred in their life before the age of 18 years. Studies have shown various methods to operationalize the ACEs variable.

ACEs collected from the CTES were operationalized as a cumulative measure based on exposure to different types of ACEs found in the questionnaire. This Total ACEs variable included personal abuses such as recurrent physical abuse, recurrent emotional abuse, and sexual abuse, as well as, household dysfunctions including having grown up with an alcoholic or drug user in home, family members in prison, mentally ill family

member, and where the mother was treated violently. For example, there were two questions regarding sexual abuse, those that answered "Yes" to at least one of the questions counted as one ACE. Other conditions such as low SES and separation from mother were excluded as they were more common occurrences. In addition to total ACEs, maltreatment ACEs that included physical, emotional, and sexual abuse and exposure to domestic violence was also examined. The ACEs variable had a range from 0-4, where 0 indicated no ACEs exposure, 1 indicated exposure to one type of ACEs, 2 indicated exposure to two types of ACEs, 3 indicated exposure to two types of ACEs, and 4 indicated exposure to four types of ACEs or more.

3.3.4 Covariates: NSAID and prescription drug usage

Additional variables collected from the questionnaire that were known to be associated with cortisol and inflammation were included in the analysis. The questionnaire included a section that asked if the participant had taken over the counter or prescribed drugs daily or on most days within the past month. The usage of any nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin or ibuprofen was included as a covariate and coded as a dichotomous variable to identify use. These drugs are known to effectively lower pain and inflammation through the inhibition of the pro-inflammatory enzyme cyclooxygenase (Ong et al., 2007). Other major prescription medication use associated with inflammation was also captured. The questionnaire included a section that asked if participants have taken any of the prescribed medicines within the past month. From the list of prescribed medications, the usage of hydrocortisone, insulin, and asthma medications were included as covariates. Usage of

prescribed insulin are known to have systemic anti-inflammatory properties (Sun et al., 2014). In addition, several studies have shown that the usage of inhaled corticosteroids and β_2 agonists in asthmatic patients lowered levels of systemic pro-inflammatory biomarker levels (Ramaraju Karthikeyan et al., 2014; Girdhar et al., 2011). A new dichotomous variable was created that indicated the participants reported usage these prescribed medications within the last month. Those that reported non usage of these drugs were coded as '0' while those that reported usage of these drugs were coded as '1'.

3.3.5 Covariates: Smoking status

Both animal and human studies have demonstrated that cigarette smoking decreases the availability of nitric oxide, a free radical largely involved with vasodilatory functions of the endothelium (Ambrose & Barua, 2004). Therefore, exposure to cigarette smoke can impair the endothelium-dependent vasodilation functions in vascular structures e.g coronary and brachial arteries. Exposure to cigarette smoke deposits activate endogenous free radicals in human tissue. In concert with free radicals from the cigarette smoke and the lack of nitric oxide, cigarette smoke increases oxidative stress in the body which leads to recruitment of immune cells such as neutrophils, monocytes, and T-cells. Furthermore, these immune cells interact with surface endothelial cells to release various pro-inflammatory cytokines to recruit more immune cells. Smoking status has been associated with elevated inflammatory markers including CRP, hsCRP, and elevated leukocyte count (Navarro et al., 2016; Pirkola et al., 2010). Because

smoking status can potentially explain findings on elevated inflammatory markers, the current study considered smoking status as a covariate. When completing the questionnaire, participants were asked to identify if they were a non-smoker, occasional smoker, or a daily smoker. For all statistical analysis, dummy variables were created so that occasional smokers and daily smokers were compared to non-smokers as the reference.

3.4 Data analysis

3.4.1 Data preparation

To prepare the data for analyses, datasets for inflammatory biomarkers, cortisol, and questionnaire were cleaned, converted, imported into Statistical Analysis System (SAS) version 9.4, and merged. Participant identification in the inflammatory biomarkers, cortisol, and questionnaire Microsoft Excel dataset were checked for consistency. These Excel files were converted into Statistical Package for the Social Sciences (SPSS) file type in preparation to importing into SAS. The datasets were imported into SAS and the files were merged based on a common, unique identifier. These procedures created one dataset for subsequent data analyses. Regression analysis assumptions for the inflammatory analytes and cortisol regression models were assessed prior to data analysis. Each independent variable including ACEs, cortisol, and covariates were assessed if they correlated with the dependent variable which were the inflammatory analytes (Figure s1-s8). The residual plots and histogram were assessed for the homoscedasticity and normality assumptions.

The biomarker multiplex and CRP assays were sensitive to a certain minimum threshold where any analyte value below this threshold or lower limit of detection were censored and listed as out of range. As the censored values are values that are known to be between zero and the lower limit of detection, values listed as out of range skew the distribution of those analytes due to cases coded as missing at the lower ranges. To manually extrapolate the censored values, the fluorescence intensity of the censored value was divided by the fluorescence intensity from the lowest standard curve value and then multiplied with the observed concentration of that same standard.

3.4.2 Statistical analyses

Hierarchical regression modeling was conducted to determine if the exposure to ACEs were associated with inflammatory markers and cortisol. Exposure to higher numbers of ACEs was expected to be associated with increased levels of pro-inflammatory biomarkers, CRP, and cytokines IFN-γ and IL-10. Cortisol, in addition to its associations with ACEs, can play a role in pro-inflammatory responses. Therefore, regression modeling was also conducted to determine if cortisol mediated the relationship between ACEs and the pro-inflammatory biomarkers, CRP, IFN-γ, and IL-10.

First, simultaneous multiple regression was used to test hypothesis 2. In Table 4.4, cortisol was regressed on ACEs and covariate variables including sex, reported usage of NSAIDs and prescription medications, and smoking status. Next, hierarchical multiple regression analysis was used to test hypothesis 1. For Tables 4.5-4.10, in Models 1 (Total ACEs) and 3 (Maltreatment ACEs), the inflammatory analytes were regressed on ACEs

and covariates. Cortisol was included in Models 2 (Total ACEs) and 4 (Maltreatment ACEs). The inflammatory analytes were regressed on ACEs, cortisol, and covariates including sex, reported usage of NSAIDs and prescription medications, and smoking status. In Table 4.11, as part of the exploratory statistical moderation analyses, Models 1 (Total ACEs) and 4 (Maltreatment ACEs) had IL-10 regressed on ACEs and covariates. Models 2 (Total ACEs) and 5 (Maltreatment ACEs) had IL-10 regressed on ACEs, cortisol, and covariates. Models 3 (Total ACEs) and 6 (Maltreatment ACEs) had IL-10 regressed on ACEs, cortisol, covariates, and the sex interaction. The interaction variables were created by calculating the product of the sex and cortisol variables (sex x cortisol) and the sex and ACEs variables (sex x ACEs). Lastly, the Sobel test was used to test hypothesis 3. Variables used in this analysis included ACEs as the independent variable, cortisol as the mediator variable, inflammatory analytes as the dependent variable, and covariates including reported usage of NSAIDs and prescription medications, and smoking status.

Chapter 4 Results

4.1 Attrition analysis of cortisol

The distribution of ACEs among total participants with and without cortisol data are reported in Table 4.1. The total sample in this study included 156 participants. A total of 106 participants had available cortisol data while 50 participants did not. The chi-square test indicated no significant difference in the proportion of ACEs score (0-4) between participants with and without cortisol data. The mean Total ACEs score after grouping to 0-4 was 1.5 ACEs (n=156) compared to the mean Maltreatment ACEs score of 1.0 ACEs (n =101). The mean Total ACEs score (0-4) among participants with cortisol data is 1.6 compared to 1.3 from participants without cortisol data. Two independent sample t-tests indicated that there is no difference in the mean Total ACEs score (0-4) between participants with and without cortisol data.

Table 4.1 Distribution of Total and Maltreatment ACEs, and ACEs among participants with and without cortisol data

	Total (n=156)	Cortisol (106)	Without cortisol (50)	P-value		Maltreatment (101)[1]
ACEs score[2], n (%)					ACEs score[3], n (%)	
0	45 (28.9)	25 (55.6)	20 (44.4)	0.31	0	48 (47.5)
1	42 (26.9)	31 (73.8)	11 (26.2)		1	25 (24.8)
2	30 (19.2)	22 (73.3)	8 (26.7)		2	16 (15.8)
3	17 (10.9)	13 (76.5)	4 (23.5)		3	8 (7.9)
4	22 (14.1)	15 (68.2)	7 (31.8)		4	4 (4.0)
ACEs score, mean (SD)	1.5 (1.4)	1.6 (1.4)	1.3 (1.4)	0.20	ACEs score, mean (SD)	1.0 (1.2)

[1]The maltreatment sample size of 101 is the final sample size used in analysis in which participants had complete data including blood and cortisol.

[2]ACEs variable includes all personal abuses and growing up in a dysfunctional household excluding marital separation or divorce.

[3]ACEs variable only includes recurrent physical abuse, recurrent emotional abuse, sexual abuse, and witnessing domestic violence.

* $p < 0.05$, two tailed.

4.2 Characteristics of demographic, covariate and main outcome variables

The chi-square test indicated that cortisol availability varied across sex (Table 4.2). Because many males did not have the required length of hair to provide adequate samples, only a total of 24 out of 68 (35.29%) males had cortisol data compared to 82 out of 88 (93.18%) for females. Participants with cortisol data were significantly younger than those without cortisol data by an average of 0.71 years. There is no difference in reported NSAID and prescription medication usage between those with and without cortisol. Participants with cortisol data had significantly higher CRP and higher IFN-γ than participants without cortisol.

Table 4.2 Distribution of demographic, covariate and main outcome variables between total participants, and participants with and without cortisol

	Total (n=156)	Cortisol (106)	Without cortisol (50)	t, χ2	df	P-value
Sex (n,%)						
Male	68	24 (35.3)	44 (64.7)	59.0	1	<.001*
Female	88	82 (93.2)	6 (6.8)			
Age (mean, SD)	22.0	21 (1.3)	22 (1.2)	3.3	154	0.001*
[1]NSAID usage (n, %)						
Yes	64	49 (76.6)	15 (23.4)	3.7	1	0.055
No	92	57 (62.0)	35 (38.0)			
[2]Prescription medication usage (n, %)						
Yes	5	4 (80.0)	1 (20.0)	0.3	1	0.557
No	151	102 (67.5)	49 (32.5)			
Smoking (n, %)						
Daily smoker	4	1 (25.0)	3 (75.0)	9.6	2	0.008*
Occasional smoker	13	5 (38.5)	8 (61.5)			
Non smoker	139	100 (71.9)	39 (28.1)			
Inflammatory analytes (mean, SD)	**Total[3] (n=147)**	**Cortisol (n=101)**	**Without cortisol (n=46)**	**t**	**df**	**P-value**
CRP	2845.3 (3533.9)	3312.3 (3845.1)	1820.0 (2469.4)	-2.8	128.7	0.006*
IL-6Rα	6517.6 (2290.0)	6642.1 (2269.6)	6244.2 (2335.7)	-1.0	145	0.331
gp130	44862.1 (9409.0)	45256.9 (8481.4)	43995.2 (11236.3)	-0.7	69.3	0.497
sTNFr1	1643.7 (738.1)	1552.8 (563.5)	1843.1 (1001.5)	1.8	58.4	0.071
sTNFr2	618.8 (197.0)	603.7 (180.1)	651.8 (228.5)	1.4	145	0.171
IFN-γ	32.4 (13.6)	34.60 (14.1)	27.6 (11.1)	-23.0	145	0.002*
IL-10	9.4 (5.6)	9.1 (5.6)	10.0 (5.7)	0.90	145	0.372

[1]Nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.

[2]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.

[3]The sample size of 147 as opposed to 156 because 9 did not have blood data.

* $p < 0.05$, two tailed.

4.3 Correlation of ACEs, cortisol and inflammatory analytes

Correlation of all inflammatory analytes with each other and in relation to ACEs, cortisol, and covariates was examined prior to running regression. CRP was positively correlated with sTNFr1, sTNFr2, and IFN-γ while IL-6Rα was positively correlated with gp130 and IFN-γ, and negatively correlated with IL-10 (Table 4.3). gp130 was negatively correlated with sTNFr1 and positively correlated with IFN-γ. sTNFr1 was positively correlated with sTNFr2. Total and maltreatment ACEs score were not correlated to any inflammatory analytes. Cortisol was not correlated with any inflammatory markers which, therefore nullifies any possible mediation of ACEs to inflammatory analytes by cortisol for this analysis. Finally, males had significantly higher levels of IL-6Ra and significantly lower levels of CRP, sTNFr1, and IL-10.

Table 4.3 Correlation matrix of all variables (n=101)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. [1]Total ACEs	-														
2. [2]Maltreatment	**0.83***	-													
3. Cortisol	-0.18	-0.17	-												
4. CRP	0.02	0.10	-0.04	-											
5. IL-6Rα	0.17	0.13	-0.19	0.18	-				-						
6. gp130	0.10	0.11	-0.15	0.13	**0.54***	-									
7. sTNFr1	0.03	0.04	-0.09	**0.33***	-0.18	**-0.20***	-								
8. sTNFr2	-0.06	-0.03	-0.08	**0.44***	0.11	-0.02	**0.75***	-							
9. IFN-γ	0.13	0.19	-0.09	**0.32***	**0.52***	**0.39***	-0.19	0.14	-						
10. IL-10	-0.04	-0.01	0.06	0.09	**-0.23***	-0.04	0.05	0.09	0.06	-					
11. [3]Sex (male)	0.04	-0.01	-0.12	**-0.21***	**0.20***	0.19	**-0.25***	-0.19	-0.03	**-0.35***	-				
12. [4]NSAIDs	0.10	0.07	0.04	-0.05	-0.18	-0.06	-0.06	**-0.21***	-0.03	0.08	0.01	-			
13. [5]Prescription medications	0.09	0.10	0.14	0.06	0.02	0.07	0.07	0.13	0.08	-0.01	-0.11	-0.01	-		
14. [6]Daily smoker	0.02	0.09	-0.03	-0.08	-0.01	-0.09	-0.04	-0.06	-0.06	0.08	0.18	0.11	-0.02	-	
15. [7]Occasional smoker	0.09	0.13	-0.03	-0.14	0.02	0.03	-0.06	-0.07	0.03	-0.15	0.09	0.15	-0.05	-0.02	-

[1]Adverse childhood experiences (total).
[2]Adverse childhood experiences (maltreatment) – only including recurrent physical abuse, recurrent emotional abuse, sexual abuse, and witnessing domestic violence.
[3]Reference category is female.
[4] Nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.
[5]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.
[6]Reported daily smoker – reference category is occasional smoker and non-smoker.
[7]Reported occasional smoker – reference category is daily smoker and non-smoker.
* $p < 0.05$, two tailed.

4.4 Regression analysis of cortisol on ACEs

Adjusted simultaneous regressions were conducted to test the association between ACEs and cortisol. Total ACEs was associated with cortisol levels after adjusting for covariates (Table 4.4 [Models 1]). For every additional ACE exposure, cortisol levels would decrease by 21.2 (pg/mg) on average. Maltreatment ACEs had a similar effect on cortisol but was not statistically significant. In addition, the interaction between ACEs and sex was non-significant (results not shown). Further exploration of a potential sex by ACE interaction was also conducted through a stratified analysis (results in appendix). This analysis showed a direction of effect was opposite across males (positive) and females (negative) suggesting a potential difference although it was nonsignificant as evidence of non-significant interaction in the full model.

Table 4.4 Results of simultaneous multiple regression in which cortisol was regressed on ACEs score, sex, medication use, and smoking status (n=101)

	Total ACEs[1]			Maltreatment ACEs[2]		
	Model 1			Model 2		
	b	p-value	95% CI	b	p-value	95% CI
Intercept	71.4	0.006	[20.56, 122.18]	57.3	0.013	[12.60, 104.48]
ACEs score	-21.2	0.039	[-41.25, -1.14]	-18.1	0.070	[-46.39, 1.88]
Sex (male)[3]	-36.9	0.270	[-102.86, 29.15]	-35.8	0.278	[-102.85, 29.87]
NSAIDs usage[4]	16.5	0.553	[-38.52, 71.52]	12.4	0.633	[-41.87, 68.42]
Prescription medication usage[5]	100.1	0.157	[-39.30, 239.40]	95.6	0.160	[-40.10, 240.45]
Daily smoker[6]	-7.3	0.959	[-285.75, 271.11]	21.7	0.941	[-270.74, 291.69]
Occasional smoker[7]	-3.2	0.960	[-129.89, 123.44]	-0.3	0.988	[-127.00, 129.02]

b (Unstandardized regression coefficients), 95% CI (95% confidence intervals).

[1]ACEs variable includes all personal abuses and growing up in a dysfunctional household excluding separation.

[2]ACEs variable only includes recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence.

[3]Reference category is female.

[4]Reported usage of nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.

[5]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.

[6]Reported daily smoker – reference category is non-smoker.

[7]Reported occasional smoker – reference category is non-smoker.

* $p < 0.05$, two tailed.

4.5 Regression analysis of inflammatory analytes on ACEs

Adjusted hierarchical multiple regressions were conducted to test if ACEs were associated with inflammatory analytes. Total maltreatment was associated with IL-6Rα (Table 4.6). Every additional exposure to a type of ACEs increased IL-6Rα levels by 284.6(pg/mL) on average. Total and maltreatment ACEs were not associated with CRP, gp130, sTNFr1, sTNFr2, IFN-γ, and IL-10 (Table 4.5, Table 4.7, Table 4.8, Table 4.9, Table 4.10, Table 4.11). In addition to ACEs score, sex, reported medication usage, and smoking covariates were not associated with CRP. Sex differences were found for both total ACEs and maltreatment ACEs in gp130, sTNFr1, and IL-10 (Table 4.7, Table 4.8, Table 4.11). In the total ACEs model and adjusting for all other variables, males had 4296.07 (pg/mL) higher levels of gp130 than females on average (Table 4.7 [Model 1]). In the maltreatment ACEs model and adjusting for all other variables, males had 4375.19 (pg/mL) higher levels of gp130 than females on average (Table 4.7 [Model 3]). In the total ACEs model and adjusting for all other variables, males had 349.37 (pg/mL) lower levels of sTNFr1 than females on average (Table 4.8 [Model 1]). In the maltreatment ACEs model and adjusting for all other variables, males had 351.51 (pg/mL) higher levels of sTNFr1 than females on average (Table 4.8 [Model 3]). In the total ACEs model and adjusting for all other variables, males had 4.91 (pg/mL) lower levels of IL-10 than females on average (Table 4.11 [Model 1]). In the maltreatment ACEs model and adjusting for all other variables, males had 4.86 (pg/mL) lower levels of IL-10 than females on average (Table 4.11 [Model 4]). Sex differences were also found for total ACEs but not in maltreatment ACEs in IL-6Rα (Table 4.6 [Model 1]). In the total ACEs

model and adjusting for all other variables, males had 1074.7 (pg/mL) higher levels of IL-6Rα than females on average.

Anti-inflammatories and steroid medications usage were also included as covariates in the regression models. In the maltreatment ACEs model and adjusting for all other variables, participants that reported using NSAID's had 72.32 (pg/mL) lower levels of sTNFr2 compared to non NSAID's users (Table 4.8 [Model 6]). The effect of cortisol on IL-10 differed between sexes. This interaction effect is discussed below.

Table 4.5 Results of hierarchal multiple regression in which CRP was regressed on ACEs score, cortisol, sex, medication use, and smoking status (n=101)

	Total ACEs[1]						Maltreatment ACEs[2]					
	Model 1			Model 2			Model 3			Model 4		
	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI
Intercept	3929.9	<.001	[2490.4, 5369.3]	4091.1	<.001	[2466.36, 5457.62]	3656.0	<.001	[2288.53, 4848.65]	3753.7	<.001	[2329.889, 4986.29]
ACEs score	-7.8	0.976	[-523.3, 507.5]	-52.1	0.939	[-558.10, 602.82]	257.8	0.245	[-276.22, 1068.75]	226.9	0.298	[-324.86, 1049.35]
Sex (male)[3]	-1735.5	0.068	[-3598.3, 127.3]	-1818.0	0.060	[-3679.69, 79.10]	-1706.8	0.073	[-3537.17, 160.82]	-1767.9	0.067	[-3612.05, 124.15]
NSAIDs[4]	-252.2	0.748	[-1805.0, 1300.7]	-214.9	0.764	[-1795.74, 1323.03]	-267.9	0.711	[-1823.84, 1248.95]	-246.8	0.732	[-1811.54, 1277.25]
Prescription medication[5]	649.8	0.743	[-3273.1, 4572.6]	861.5	0.687	[-3173.17, 4795.48]	527.1	0.833	[-3491.75, 4324.79]	690.2	0.776	[-3395.93, 4535.27]
Daily smoker[6]	-1532.5	0.700	[-9410.1, 6345.1]	-1513.1	0.691	[-9457.83, [6295.08]	-2068.6	0.614	[-9834.70, 5835.75]	-2031.6	0.618	[-9850.17, 5883.24]
Occasional smoker[7]	-1992.9	0.270	[-5558.5, 1572.7]	-2009.5	0.262	[-5620.68, 1545.62]	-2199.3	0.209	[-5840.35, 1292.92]	-2199.8	0.211	[-5853.04, 1308.69]
Cortisol	-	-	-	-2.2	0.486	[-7.84, 3.75]	-	-	-	-1.7	0.597	[-7.26 4.20]

b (Unstandardized regression coefficients), 95% CI (95% confidence intervals).

[1]ACEs variable includes all personal abuses and growing up in a dysfunctional household excluding separation.

[2]ACEs variable only includes recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence.

[3]Reference category is female.

[4]Reported usage of nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.

[5]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.

[6]Reported daily smoker – reference category is non-smoker.

[7]Reported occasional smoker – reference category is non-smoker.

* $p < 0.05$, two tailed.

Table 4.6 Results of hierarchal multiple regression in which IL-6Rα was regressed on ACEs score, cortisol, sex, medication use, and smoking status (n=101)

	Total ACEs[1]						Maltreatment ACEs[2]					
	Model 1			Model 2			Model 3			Model 4		
	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI
Intercept	6261.4	<.001	[5423.33, 7069.90]	6425.0	<.001	[5552.78, 7259.12]	6435.6	<.001	[5749.72, 7245.53]	6571.8	<.001	[5874.11, 7410.50]
ACEs score	284.6	0.050	[0.63, 650.53]	239.8	0.099	[-52.86, 609.38]	304.9	0.160	[-112.57, 673.27]	261.9	0.263	[-172.05, 622.75]
Sex (male)[3]	1158.4	0.035	[86.22, 2225.25]	1074.7	0.050	[1.36, 2145.53]	1142.8	0.038	[62.60, 2223.24]	1057.6	0.056	[-27.71, 2133.20]
NSAIDs[4]	-897.4	0.048	[-1789.67, -6.65]	-859.6	0.058	[-1750.86, 28.21]	-836.2	0.065	[-1741.19, 54.15]	-806.8	0.075	[-1703.94, 82.53]
Prescription medication[5]	442.9	0.748	[-1892.01, 2623.75]	657.7	0.608	[-1683.59, 2862.05]	421.1	0.729	[-1883.93, 2683.06]	648.4	0.577	[-1646.45, 2940.73]
Daily smoker[6]	-779.2	0.806	[-5069.78, 3953.07]	-759.5	0.800	[-5067.76, 3918.34]	-1060.0	0.742	[-5338.66, 3817.17]	-1008.4	0.749	[-5284.74, 3815.03]
Occasional smoker[7]	247.0	0.867	[-1879.01, 2225.67]	230.1	0.872	[-1877.85, 2210.11]	107.3	0.886	[-1932.45, 2235.33]	106.6	0.883	[-1917.14, 2225.00]
Cortisol	-	-	-	-2.2	0.183	[-5.54, 1.07]	-	-	-	-2.4	0.142	[-5.78, 0.84]

b (Unstandardized regression coefficients), 95% CI (95% confidence intervals).

[1]ACEs variable includes all personal abuses and growing up in a dysfunctional household excluding separation.

[2]ACEs variable only includes recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence.

[3]Reference category is female.

[4]Reported usage of nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.

[5]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.

[6]Reported daily smoker – reference category is non-smoker.

[7]Reported occasional smoker – reference category is non-smoker.

* $p < 0.05$, two tailed.

Table 4.7 Results of hierarchal multiple regression in which gp130 was regressed on ACEs score, cortisol, sex, medication use, and smoking status (n=101)

	Total ACEs[1]						Maltreatment ACEs[2]					
	Model 1			Model 2			Model 3			Model 4		
	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI
Intercept	44031.0	<.001	[40391.00, 46668.00]	44621.0	<.001	[40794.00, 47315.00]	43708.0	<.001	[40930.00, 46564.00]	44123.0	<.001	[41266.00, 47079.00]
ACEs score	316.6	0.279	[-558.69, 1919.03]	155.2	0.413	[-741.20, 1789.7]	851.3	0.213	[-545.08, 2414.91]	720.3	0.310	[-730.55, 2276.97]
Sex (male)[3]	4597.6	0.026	[561.50, 8716.37]	4296.1	0.037	[270.60, 8465.08]	4634.7	0.026	[583.51, 8721.92]	4375.2	0.036	[299.15, 8476.04]
NSAIDs[4]	-1001.2	0.530	[-4477.19, 2320.43]	-865.0	0.578	[-4356.62, 2442.57]	-960.2	0.561	[-4374.68, 2387.80]	-870.6	0.600	[-4276.96, 2483.02]
Prescription medication[5]	3925.3	0.402	[-4960.16, 12256.00]	4699.0	0.319	[-4302.46, 13070.00]	3663.9	0.419	[-5085.61, 12117.00]	4356.7	0.334	[-4435.47, 12922.00]
Daily smoker[6]	-10642.0	0.228	[-27714.00, 6684.51]	-10571.0	0.225	[-27740.00, 6602.52]	-12001.0	0.195	[-28584.00, 5902.53]	-11843.0	0.197	[-28482.00, 5951.88]
Occasional smoker[7]	492.3	0.952	[-7584.75, 8064.01]	431.5	0.956	[-7595.67, 8027.48]	-67.5	0.990	[-7900.44, 7798.21]	-69.8	0.991	[-7880.71, 7793.13]
Cortisol	-	-	-	-8.1	0.250	[-19.99, 5.27]	-	-	-	-7.2	0.253	[-19.80, 5.27]

b (Unstandardized regression coefficients), 95% CI (95% confidence intervals).

[1]ACEs variable includes all personal abuses and growing up in a dysfunctional household excluding separation.

[2]ACEs variable only includes recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence.

[3]Reference category is female.

[4]Reported usage of nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.

[5]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.

[6]Reported daily smoker – reference category is non-smoker.

[7]Reported occasional smoker – reference category is non-smoker.

* $p < 0.05$, two tailed.

Table 4.8 Results of hierarchal multiple regression in which sTNFr1 was regressed on ACEs score, cortisol, sex, medication use, and smoking status (n=101)

	Total ACEs[1]						Maltreatment ACEs[2]					
	Model 1			Model 2			Model 3			Model 4		
	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI
Intercept	1638.2	<.001	[1430.23, 1848.78]	1678.1	<.001	[1461.57, 1895.60]	1659.4	<.001	[1452.26, 1828.58]	1692.0	<.001	[1478.03, 1865.71]
ACEs score	10.0	0.806	[-72.37, 92.83]	-0.9	0.974	[-85.60, 82.85]	-4.2	0.737	[-82.10, 115.60]	-14.5	0.925	[-95.48, 105.07]
Sex (male)[3]	-329.0	0.018	[-601.11, -57.38]	-349.4	0.013	[-622.12, -76.73]	-331.1	0.018	[-600.51, -56.94]	-351.5	0.013	[-620.96, -75.69]
NSAIDs[4]	-63.3	0.582	[-289.69, 163.54]	-54.1	0.636	[-280.31, 172.22]	-60.4	0.587	[-287.86, 163.81]	-53.4	0.630	[-280.28, 170.50]
Prescription medication[5]	109.8	0.710	[-466.17, 681.70]	162.1	0.578	[-415.58, 740.67]	115.9	0.720	[-470.27, 678.70]	170.3	0.589	[-420.72, 736.77]
Daily smoker[6]	63.0	0.902	[-1075.64, 1217.92]	67.8	0.907	[-1075.74, 1210.00]	83.6	0.924	[-1096.10, 1207.33]	95.9	0.916	[-1086.84, 1209.32]
Occasional smoker[7]	-73.7	0.774	[-597.36, 446.02]	-77.8	0.768	[-597.35, 442.48]	-66.8	0.757	[-606.19, 442.34]	-67.0	0.758	[-603.98, 441.21]
Cortisol	-	-	-	-0.5	0.199	[-1.39, 0.29]	-	-	-	-0.6	0.205	[-1.37, 0.30]

b (Unstandardized regression coefficients), 95% CI (95% confidence intervals).
[1]ACEs variable includes all personal abuses and growing up in a dysfunctional household excluding separation.
[2]ACEs variable only includes recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence.
[3]Reference category is female.
[4]Reported usage of nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.
[5]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.
[6]Reported daily smoker – reference category is non-smoker.
[7]Reported occasional smoker – reference category is non-smoker.
* $p < 0.05$, two tailed.

Table 4.9 Results of hierarchal multiple regression in which sTNFr2 was regressed on ACEs score, cortisol, sex, medication use, and smoking status (n=101)

	Total ACEs[1]						Maltreatment ACEs[2]					
	Model 1			Model 2			Model 3			Model 4		
	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI
Intercept	665.0	<.001	[598.89, 730.66]	677.6	<.001	[608.63, 745.29]	660.0	<.001	[597.55, 716.22]	669.3	<.001	[605.00, 727.38]
ACEs score	-6.9	0.570	[-33.46, 18.55]	-10.3	0.409	[-37.60, 15.44]	-6.5	0.800	[-35.15, 27.19]	-9.5	0.638	[-39.17, 24.14]
Sex (male)[3]	-77.9	0.074	[-163.39, 7.79]	-84.3	0.055	[-169.96, 1.78]	-77.5	0.077	[-162.93, 8.48]	-83.3	0.059	[-169.09, 3.04]
NSAIDs[4]	-72.8	0.045	[-144.24, -1.55]	-69.9	0.054	[-141.33, 1.16]	-74.3	0.041	[-145.57, -3.14]	-72.3	0.047	[-143.39, -1.09]
Prescription medication[5]	104.0	0.249	[-75.02, 286.35]	120.5	0.184	[-59.29, 304.78]	104.2	0.260	[-77.68, 284.64]	119.8	0.198	[-63.29, 302.10]
Daily smoker[6]	1.3	0.982	[-365.27, 356.79]	2.8	0.976	[-365.35, 354.38]	6.3	0.990	[-365.60, 360.77]	9.8	0.997	[-363.17, 361.67]
Occasional smoker[7]	-10.1	0.918	[-172.74, 155.74]	-11.4	0.913	[-172.76, 154.66]	-7.4	0.908	[-175.03, 155.62]	-7.4	0.909	[-174.52, 155.42]
Cortisol	-	-	-	-0.8	0.204	[-0.44 0.09]	-	-	-	-0.2	0.235	[-0.42 0.10]

b (Unstandardized regression coefficients), 95% CI (95% confidence intervals).
[1]ACEs variable includes all personal abuses and growing up in a dysfunctional household excluding separation.
[2]ACEs variable only includes recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence.
[3]Reference category is female.
[4]Reported usage of nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.
[5]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.
[6]Reported daily smoker – reference category is non-smoker.
[7]Reported occasional smoker – reference category is non-smoker.
* $p < 0.05$, two tailed.

Table 4.10 Results of hierarchal multiple regression in which IFN-γ was regressed on ACEs score, cortisol, sex, medication use, and smoking status (n=101)

	Total ACEs[1]						Maltreatment ACEs[2]					
	Model 1			Model 2			Model 3			Model 4		
	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI
Intercept	33.3	<.001	[27.47, 38.17]	34.0	<.001	[27.88, 39.04]	32.7	<.001	[27.94, 37.47]	33.2	<.001	[28.23, 38.10]
ACEs score	0.9	0.235	[-0.84, 3.39]	0.7	0.323	[-1.08, 3.25]	2.0	0.066	[-0.16, 4.85]	1.9	0.095	[-0.38, 4.72]
Sex (male)[3]	-0.2	0.954	[-7.16, 6.75]	-0.6	0.880	[-7.55, 6.48]	-0.2	0.975	[-7.00, 6.77]	-0.5	0.910	[-7.34, 6.55]
NSAIDs[4]	-1.0	0.722	[-6.84, 4.76]	-0.8	0.761	[-6.71, 4.92]	-0.8	0.747	[-6.65, 4.79]	-0.7	0.775	[-6.57, 4.91]
Prescription medication[5]	5.5	0.496	[-9.63, 19.73]	6.4	0.429	[-8.92, 20.81]	4.9	0.544	[-10.09, 19.00]	5.6	0.482	[-9.49, 19.98]
Daily smoker[6]	-8.8	0.580	[-37.54, 21.12]	-8.7	0.577	[-37.67, 21.11]	-11.9	0.479	[-39.60, 18.72]	-11.7	0.483	[-39.56, 18.88]
Occasional smoker[7]	2.2	0.790	[-11.55, 15.14]	2.1	0.794	[-11.60, 15.14]	0.9	0.901	[-12.44, 14.11]	0.9	0.900	[-12.47, 4.15]
Cortisol	-	-	-	-0.1	0.414	[-0.03, 0.01]	-	-	-	-0.1	0.465	[-0.03, 0.01]

b (Unstandardized regression coefficients), 95% CI (95% confidence intervals).

[1]ACEs variable includes all personal abuses and growing up in a dysfunctional household excluding separation.

[2]ACEs variable only includes recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence.

[3]Reference category is female.

[4]Reported usage of nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.

[5]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.

[6]Reported daily smoker – reference category is non-smoker.

[7]Reported occasional smoker – reference category is non-smoker.

* $p < 0.05$, two tailed.

Table 4.11 Results of hierarchal multiple regression in which IL-10 was regressed on ACEs score, cortisol, sex, medication use, and smoking status (n=101)

	Total ACEs[1]									Maltreatment ACEs[2]								
	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI	b	p-value	95% CI
Intercept	10.2	<.001	[8.25, 12.17]	10.2	<.001	[8.15, 12.25]	10.6	<.001	[8.49, 12.55]	9.9	<.001	[8.15, 11.68]	9.9	<.001	[8.05, 11.72]	10.1	<.001	[8.28, 11.91]
ACEs score	-0.2	0.565	[-1.0, 0.55]	-0.2	0.581	[-1.02, 0.57]	-0.3	0.382	[-1.14, 0.44]	-0.1	0.907	[-0.98, 0.87]	-0.	0.928	[-0.99, 0.90]	-0.1	0.757	[-1.08, 0.79]
Sex (male)[3]	-4.9	0.001	[-7.45, -2.36]	-4.9	0.001	[-7.48, -2.33]	-7.9	<.001	[-11.66, -4.17]	-4.9	0.001	[-7.43, -2.33]	-4.9	0.001	[-7.44, -2.29]	-7.7	<.001	[-11.47, -3.99]
NSAIDs usage[4]	1.0	0.336	[-1.09, 3.15]	1.0	0.340	[-1.10, 3.17]	1.0	0.344	[-1.09, 3.09]	1.0	0.359	[-1.13, 3.10]	1.0	0.365	[-1.15, 3.11]	0.9	0.381	[-1.17, 3.02]
Prescription medication usage[5]	-1.2	0.661	[-6.56, 4.18]	-1.2	0.663	[-6.66, 4.25]	-1.2	0.670	[-6.50, 4.20]	-1.3	0.635	[-6.68, 4.09]	-1.4	0.626	[-6.82, 4.13]	-1.3	0.627	[-6.70, 4.06]
Daily smoker[6]	7.6	0.173	[-3.31, 18.14]	7.6	0.176	[-3.37, 18.20]	10.2	0.072	[-0.89, 20.77]	7.4	0.177	[-3.40, 18.19]	7.4	0.180	[-3.47, 18.24]	10.0	0.076	[-1.05, 20.81]
Occasional smoker[7]	-3.2	0.196	[-8.08, 1.68]	-3.2	0.199	[-8.10, 1.71]	-5.1	0.053	[-10.14, 0.06]	-3.3	0.188	[-8.19, 1.63]	-3.3	0.190	[-8.22, 1.66]	-5.0	0.055	[-10.16, 0.11]
Cortisol	-	-	-	0.1	0.974	[-0.01, 0.01]	-0.1	0.917	[-0.01, 0.01]	-	-	-	0.1	0.894	[-0.01, 0.01]	0.1	0.978	[-0.01, 0.01]
Sex*Cortisol[8]	-	-	-	-	-	-	0.3	0.033	[0.03, 0.66]	-	-	-	-	-	-	0.3	0.041	[0.01, 0.64]

b (Unstandardized regression coefficients), 95% CI (95% confidence intervals).

[1]ACEs variable includes all personal abuses and growing up in a dysfunctional household excluding separation.

[2]ACEs variable only includes recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence.

[3]Reference category is female.

[4]Reported usage of nonsteroidal anti-inflammatory drug – includes aspirin or ibuprofen.

[5]Prescription medication usage – includes insulin, inhaled corticosteroids, or β_2 agonists.

[6]Reported daily smoker – reference category is non-smoker.

[7]Reported occasional smoker – reference category is non-smoker.

* $p < 0.05$, two tailed.

4.6 Moderation analyses

Sex was independently associated with CRP, IL-6Rα, sTNFr1, and IL-10. Therefore, additional interaction variables were included in the inflammatory analyte regression models to test for potential moderating effects of respondent sex and ACEs score and on cortisol (Tables 4.5-4.10 [Models 1 and 3] and Tables 4.11 [Models 1 and 4]). For the cortisol regression analysis, as it was the dependent variable, only sex by ACEs score was included in the regression model (Table 4.4 [Model 1 and 2]). Fourteen models tested for sex interaction on inflammatory markers. Out of fourteen models, a significant sex by cortisol interaction was only found in both the Total ACEs and Maltreatment ACEs in the regression models for IL-10 (Table 4.11 [Model 1 and 4]) indicating that the relationship between cortisol and IL-10 was moderated by sex. For every 1 (pg/mg) increase in cortisol, IL-10 is increased by 0.34 (pg/mL) on average for males compared to females (Table 4.11 [Model 1 and 4]). To present this finding in figure form, first the regression equation was extrapolated from Table 4.11 [Model 1]. Next, the mean and standard deviation of IL-10 were used in the regression slopes to plot the interaction between males and females (Figure 4.1). No additional interactions between sex and either ACEs or cortisol were found to be significant. To further illustrate the sex interactions, stratified analyeis of the differences across sex by ACEs and cortisol on IL-6Rα and CRP are presented in the appendix. Due to the significant independent effects of sex on CRP, IL-6Rα, sTNFr1, and IL-10, further investigation through these stratified analyses by sex were conducted to investigate the non-significant interactions (results in appendix).

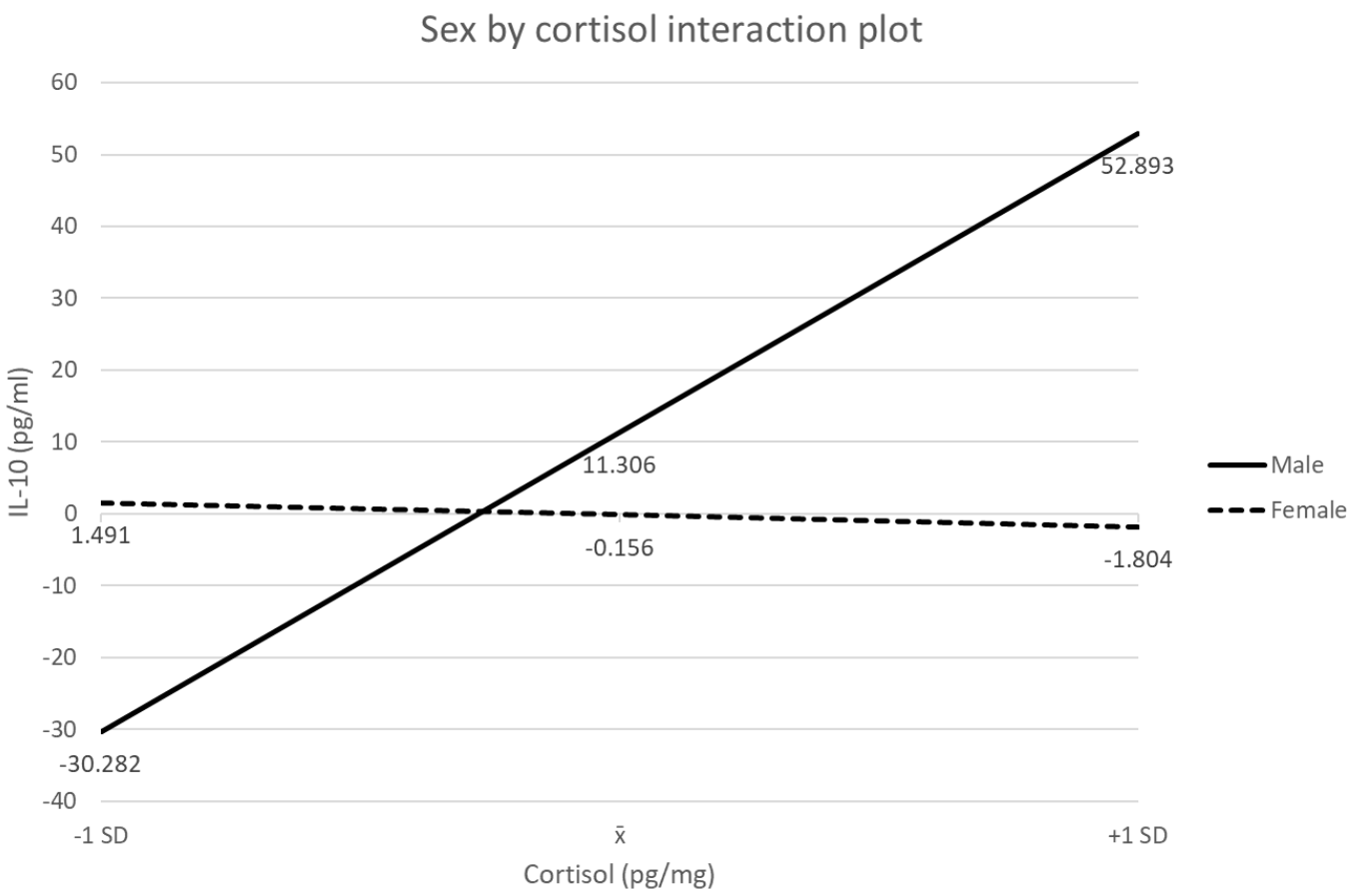

Figure 4.1 Effect of cortisol on IL-10 stratified by sex on Total ACEs

Note: See Table 4.11 Model 3

4.7 Mediation analysis of ACEs to inflammatory analytes via cortisol

Sobel tests were conducted to test the significance of cortisol as a mediator between ACEs and inflammatory analytes. As expected, based on the regression analyses above, there was no significant mediation for either the unadjusted and adjusted total ACEs score on all inflammatory analytes through cortisol (Table 4.12 & 4.13). There was also no significant mediation for either the unadjusted and adjusted maltreatment ACEs score on all inflammatory analytes through cortisol (Table 4.14 & 4.15).

Table 4.12 Unadjusted Total ACEs mediation models via Sobel test

Independent variable	Mediator	Dependent variable	Total effect mediated	P-value
ACEs score[1]	Cortisol	CRP	113.3%	0.674
ACEs score[1]	Cortisol	IL-6Rα	20.7%	0.201
ACEs score[1]	Cortisol	gp130	76.1%	0.246
ACEs score[1]	Cortisol	sTNFr1	60.8%	0.413
ACEs score[1]	Cortisol	sTNFr2	28.6%	0.395
ACEs score[1]	Cortisol	IFN-γ	17.6%	0.723
ACEs score[1]	Cortisol	IL-10	28.0%	0.628

Notes. [1]Total Adverse childhood experiences. * $p < 0.05$, two tailed.

Table 4.13 Adjusted Total ACEs mediation models via Sobel test

Independent variable	Covariates	Mediator	Dependent variable	Total effect mediated	P-value
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	CRP	513.4%	0.478
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	IL-6Rα	14.4%	0.262
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	gp130	46.8%	0.283
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	sTNFr1	100.4%	0.277
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	sTNFr2	45.8%	0.277
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	IFN-γ	20.4%	0.420
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	IL-10	0.5%	0.987

Notes. [1] Total Adverse childhood experiences. [a]C – Sex, [b]C – NSAID usage, [c]C – Prescription medication usage, [d]C – Daily smoker, and [e]C – Occasional smoker. * $p < 0.05$, two tailed.

Table 4.14 Unadjusted Maltreatment ACEs mediation models via Sobel test

Independent variable	Mediator	Dependent variable	Total effect mediated	P-value
ACEs score[1]	Cortisol	CRP	7.6%	0.766
ACEs score[1]	Cortisol	IL-6Rα	16.5%	0.239
ACEs score[1]	Cortisol	gp130	18.4%	0.309
ACEs score[1]	Cortisol	sTNFr1	142.4%	0.404
ACEs score[1]	Cortisol	sTNFr2	27.5%	0.437
ACEs score[1]	Cortisol	IFN-γ	5.8%	0.543
ACEs score[1]	Cortisol	IL-10	149.9%	0.597

Notes. [1]Adverse childhood experiences – only including recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence. * $p < 0.05$, two tailed.

Table 4.15 Adjusted Maltreatment ACEs mediation models via Sobel test

Independent variable	Covariates	Mediator	Dependent variable	Total effect mediated	P-value
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	CRP	11.1%	0.578
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	IL-6Rα	13.0%	0.280
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	gp130	14.2%	0.346
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	sTNFr1	225.5%	0.296
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	sTNFr2	41.9%	0.325
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	IFN-γ	6.6%	0.495
ACEs score[1]	[a]C, [b]C, [c]C, [d]C, [e]C	Cortisol	IL-10	17.7%	0.894

Notes. [1]Adverse childhood experiences – only including recurrent physical abuse, recurrent emotional abuse, sexual abuse, and domestic violence. [a]C – Sex, [b]C – NSAID usage, [c]C – Prescription medication usage, [d]C – Daily smoker, and [e]C – Occasional smoker. * $p < 0.05$, two tailed.

Chapter 5 Discussion

Research has highlighted the link between ACEs and physiological measures associated for diseases later in life (Miller et al., 2011; Steptoe et al., 2007; Ehlert, 2013; Kuhlman et al., 2017). More importantly, biological embedding has been the proposed mechanism explaining how ACEs can contribute to the development of diseases of later life through chronic inflammation (Miller et al., 2011). Through specific mechanisms such as epigenetic pathways and post-translational modifications, adaptations to stress in the early years of life gets embedded into immune cells that initiate and maintain inflammation. These genetic modifications are carried forward throughout the lifespan and ultimately contribute to low grade chronic inflammation.

With the established link between social determinants of health such as low SES and ACEs and elevated pro-inflammatory markers, the first study objective investigated the association between ACEs and increased inflammatory analytes. Inflammatory analytes included in the current study were one acute phase protein (CRP), four soluble receptors (IL-6Rα, gp130, sTNFr1, and sTNFr2), and two cytokines (IFN-γ and IL-10). CRP is an acute phase protein that is often used to measure systemic inflammation and has been linked to social determinants such as low SES (Taylor et al., 2006; Friedman et al., 2015) and ACEs (Hostinar et al., 2015). In contrast, total ACEs or maltreatment ACEs were not associated with CRP in the current study. Because CRP is a marker of acute inflammatory processes such as a bacterial infection or the presence of chronic inflammatory conditions such as cardiovascular disease, a plausible explanation as to

why elevated CRP was not associated with increased exposure to ACEs is the sample age between studies. Taylor et al. (2006)'s sample had an average age of 40 years, Friedman et al. (2015)'s sample had an average age of 54.5 years, while Hostinar et al. (2015)'s sample had an average age of 57 years. Interestingly, in comparison to Total ACEs, Maltreatment ACEs had a much larger regression coefficient that was closer to statistical significance (Table 4.5). Total ACEs but not Maltreatment ACEs were associated with IL-6Rα. However, Total and Maltreatment ACEs were not associated with any of the other pro-inflammatory analytes. IL-6Rα was correlated with gp130 but both Total and Maltreatment ACEs were not associated with gp130. Because the 95% CI would likely narrow and exclude zero with an increased sample, the regression models of ACEs with gp130 would likely achieve statistical significance with a full NLHS sample calculated to be adequately powered (Table 4.6). As studies have shown that IL-6Rα and gp130 are highly correlated to IL-6, the findings in the current study are somewhat consistent with the findings from Hostinar et al. (2015) and Kiecolt-Glaser et al. (2011) where they found positive associations between the cumulative ACEs exposure and IL-6. The current study also investigated pro- and anti-inflammatory cytokines, specifically IL-10 and IFN-γ, which are less studied compared to IL-6 and TNFα in this context. ACEs were not associated with either IFN-γ nor IL-10. However, the regression model for IFN-γ were just above the level of statistical significance but only in the Maltreatment ACEs model. In the context of the current study, IFN-γ is a cytokine secreted by T_h1 cells to skew macrophages into an M1 response. An M1 response is known to promote release of pro-inflammatory cytokines such as IL-6 and TNFα. Interestingly, IFN-γ was positively

correlated to IL-6Rα and gp130, which was expected, but not sTNFr1 and sTNFr2, which was unexpected. Even though most regression analysis between ACEs and inflammatory analytes were not statistically significant, the confidence intervals suggest that the effect of an accumulation of ACEs on CRP, gp130, and IFN-γ may have been statistically significant if the full sample were analyzed instead of the current pilot sample. Surprisingly, the combination of these analytes that included IL-6Rα, gp130, and IFN-γ resemble a pro-inflammatory response that is characterized by classically activated macrophages that promotes inflammation involving phagocytosis. Although cytokines are pleiotropic, meaning one cytokine can exert many different types of effects on different types of cells as well as the ability to be redundant, meaning that other cytokines or combination of cytokines can also elicit a similar inflammatory response of interest. Therefore, it can be difficult to link specific immune cells involved with exposure to social determinants of health such as ACEs through specific groups of cytokines as cytokines are often a part of a large complex network of molecules involved in routine immune signalling.

The younger age of the sample used in this study compared to previous studies may also have minimized the effect size of ACEs on the inflammatory analytes. For example, studies that investigated both cortisol and inflammatory cytokines generally used older samples compared to the current study. The study by Miller et al. (2009) investigated early life SES on inflammation and included a sample of adults with an average age of 34 years which is about a decade older than the participants in the current study. The study by Friedman et al. (2015) and Hostinar et al. (2015) also

included a sample of adults with an average age of 54.5 and 57 years respectively. The study by Kiecolt-Glaser et al. (2011) included a much older sample with an average age of 69.7 years. The levels of chronic inflammation identified in these studies are considered low grade and, as such, it may take many years to develop in cells that have been programmed to be more pro-inflammatory. Moreover, stressors occurring throughout life accentuate this inflammation so that it becomes chronic, such that over the adult life span, the body increasingly responds to stress in a way that is maladaptive.

Different inflammation measures might also account for this difference as previous studies used other serum markers such as IL-6, fibrinogen, E-selectin, and Intercellular Adhesion Molecule-1 as opposed to receptors of pro-inflammatory cytokines (Hostinar et al., 2015). In addition to the age difference between the current study and previous work, the link between cortisol and pro-inflammatory receptors with respect to ACEs have not been studied as extensively as their respective cytokines. The soluble receptors IL-6Rα, gp130, sTNFr1, and sTNFr2 are often generated from cell membrane shedding or secreted from the cell from differential messenger RNA splicing as opposed to reliance on NF-κB activity. Coupled with the fact that cortisol supresses inflammation through inhibition of NF-κB activity, the association between cortisol and soluble receptors may not be as strong as the connection with pro-inflammatory cytokines. Even though several other studies show high correlations between pro-inflammatory cytokines and their respective receptors (Safranow et al., 2009; Pai et al., 2004; Neirynck et al., 2015), the receptors are not fully exclusive to the cytokines that

operate through them which may explain the difference in inflammatory findings between studies.

Because ACEs and other social determinants have been linked to both elevated and lowered cortisol levels across different age groups, another objective of the current study was to investigate the relationship between ACEs and cortisol (Cicchetti & Rogosch, 2001). In the current study, ACEs score was found to be independently associated with lowered cortisol levels (Table 4.4, Model 1 and 2). This result was opposite of what was originally hypothesized where a higher number of ACEs exposure led to increases in cortisol levels similar to previous work on children (Cicchetti & Rogosch, 2001).

Interestingly, the findings of the current study suggest that the long-term effects of ACEs on cortisol levels identified among middle to older aged adults are also present among young adults. The findings from the current study reflects the cortisol findings from the meta-analysis of cortisol by Miller et al. (2007). In Miller et al. (2007), lower cortisol levels were found in participants where the initial stimulus was no longer present. In addition, this relationship also resembles the findings from Yehuda and colleagues (2002) where holocaust survivors diagnosed with posttraumatic stress disorder presented abnormally low cortisol levels. In contrast, other studies that have investigated early childhood adversity have shown that social determinants such as low SES and ACEs were associated with elevated cortisol levels when examined in childhood. In comparison, middle to older adults that have reported previous ACEs exposure had lower cortisol. Cortisol levels appear to elevate in response to traumatic events, an

indication of allostasis. In response to external threats or stimuli, there is an internal response that becomes activated to better adapt oneself to deal with future threats. Shaped by evolution, physiological responses such as elevating cortisol levels are intended to maximize survival. Fast forward to older populations with a history of ACEs, cortisol levels are much lower and indicate allostatic load which is a consequence of having allostatic mechanisms chronically worked over time. Taken together, there appears to be an inflection point among those exposed to ACEs, where these allostatic mechanisms work first by increasing cortisol levels but begins to wear out. It is believed that after the inflection point, allostatic load takes over and cortisol levels drop significantly below normal ranges. At what age this inflection point manifests is a question for further investigation.

As part of the physiological stress response, cortisol is also largely involved in the regulation of inflammatory processes such as suppression. Circulating cortisol supress inflammation by binding to glucocorticoid receptors on immune cells. Due to this vital role of cortisol on inflammation and previous work linking ACEs to both cortisol levels and inflammation, one of the main objectives of the study was to investigate if systemic inflammation is mediated by cortisol levels among those with a history of ACEs. The Sobel test was used to test if ACEs transmitted its effect to each of the inflammatory analytes through cortisol. Cortisol was not associated with any of the inflammatory analytes as was expected from the regression analysis. As a result, cortisol did not mediate the relationship between ACEs and inflammation. Cortisol was hypothesized to mediate the relationship between ACEs and inflammation because ACEs are known to

influence both cortisol and inflammatory analytes in addition to cortisol being a primary suppressor of inflammation.

Overall, the relationship between cortisol and the various markers of inflammation are inconclusive as the findings are not consistent with the biological embedding theory. Because there is evidence that increased exposure to ACEs contributes to increased pro-inflammatory analytes in the current study, these findings may represent a transition period or point of inflection where cortisol levels are recalibrating from higher levels in childhood to lower levels in later adulthood which may currently foster an environment to amplify pro-inflammatory tendencies in immune cells.

Moreover, while sex differences for the soluble receptors were not reported in previous literature, sex was independently associated with multiple inflammatory analytes, but the effects varied across analytes. Specifically, males had significantly higher levels of IL-6Rα and gp130 but females had significantly higher levels of sTNFr1 and IL-10. There also appeared to be a moderating effect of sex on the relationship between cortisol and IL-10. A 1 pg/mg increase in cortisol corresponded to a 0.34 pg/mL increase in IL-10 on average in males compared to females. While no other test of interaction was statistically significant, we cannot conclusively rule out differences across males and females as evidence by the stratified analysis. For example, the ACE by sex interaction for cortisol, while non-significant, showed opposite direction of effects between males and females in the partial correlations. This indicates that a sex difference may exist but it was undetectable due to the lack of a sufficient sample.

Finally, smoking patterns and medication usage were included in the analysis as covariates. Smoking patterns were not associated with inflammatory analytes or cortisol. Anti-inflammatory and cortisol-related medications, (e.g., inhaled corticosteroids and β_2 agonists) were included as covariates to account for substances that can directly influence inflammatory analytes and cortisol levels. Out of all the regression analyses, only one model had a significant effect. In the Maltreatment ACEs model, compared to participants reported no NSAIDs usage, those that reported usage were found to have an average of 72.3 pg/mL less sTNFr2. Other than coincidental chance, this result reflected the effects that NSAIDs can have on lowering inflammation and consequently the associating inflammatory analytes.

5.1 Limitations, strengths, and future directions

The current study is one of the first to investigate associations between ACEs and inflammatory analytes and cortisol in a biological embedding model among young adults. Based on previous work in biological embedding, relevant physiological markers specifically IL-6Rα, gp130, sTNFr1, sTNFr2, IFN-γ, and IL-10 were included as measures for inflammation while cortisol was included as a measure of chronic stress reactivity. Overall, there were four issues in relation to the current study that may limit or strengthen these results that require further discussion.

First, most of the ACEs literature examining this relationship with similar physiological measures have coded ACEs cumulatively (Hostinar et al., 2015; Danese et al., 2007). Unlike previous work, the current study employed two different

operationalizations of the accumulation of ACEs, one combining maltreatment with household dysfunction and one examining only maltreatment. Using the Total ACEs which accounted for both maltreatment and household dysfunction allowed for the comparison of this study with studies that followed how it was measured in the original ACEs study by Felliti et al. (1998). The second method examined Maltreatment ACEs where only abuse, neglect, and witnessing domestic violence were included to assess how these ACEs which are commonly perceived of as being much more severe influence a pro-inflammatory response. Therefore, the current study was able assess differences across categories of ACES but, interestingly, found little difference across them. Further work could also examine the effect of the third category of ACEs on cortisol and inflammation that include event and experiences outside of the home such as exposure to natural disasters, bullying, and community-level toxic environments.

Second, because the current study included a pilot sample, a series of analyses were conducted to test for statistical power. A post hoc test for the cortisol regression model with an α error of 0.05, the calculated effect size of 0.082 from an R^2 value of 0.0758, and total sample size of 101 with 6 predictor values yielded a power of 0.52. An *a priori* test using the same effect size indicated that the cortisol regression model with a power of 0.80 and at an α error of 0.05 would require 173 participants. Similarly, a post hoc test conducted on CRP revealed a power of 0.43 and an *a priori* test powered at 0.80 revealed that a total of 204 participants with CRP data was needed. In addition, with a Pearson correlation of 0.19 between cortisol and IL-6Rα (Table 4.3), a minimum sample size of 346 participants was needed to order to run an analysis that is powered at 0.80.

Future analyses conducted on the specific physiological measures in the current study should include an adequately powered sample to provide a sound test to detect any associations.

Third, unlike previous research on ACEs, the analytes assessed in the current study are the soluble receptors of the more commonly studied cytokines in previous work, IL-6 and TNFα. These receptors are commonly analyzed together with the cytokines that operate through these receptors in clinical populations (Safranow et al., 2009). Therefore, the difference between using the receptor or the cytokine was expected to be minimal. Previous research has identified concrete associations between ACEs and elevated cytokine levels but there has been no research examining ACEs and soluble receptors. While elevations in soluble receptors are prevalent in clinical populations on non-ACEs studies, more studies are needed to establish the relationship between ACEs and soluble receptors in healthy populations. However, ACEs were associated with IL-6Rα and is therefore one of the first studies to link ACEs with this specific soluble receptor. Given so, future analyses could examine both the cytokine and the cytokine receptors to better identify the relationship between both cytokines and cytokine receptors and between them and ACEs.

Finally, the current study provided a more stable measure of chronic cortisol compared to previous work. Hair samples provide a 3-month retrospective average level of cortisol that is a more precise indicator of chronic stress response which is less influenced by acute stressors and variations in circadian rhythm compared to other methods such as salivary cortisol. Most previous work measured cortisol from saliva,

urine, or serum (Chen et al., 2010; Cicchetti & Rogosch, 2001; van der Vegt et al., 2009) providing a point estimate that is not representative of the overall levels of cortisol (Meyer et al., 2014). Some studies attempted to go beyond point estimates and included multiple point estimate measurements throughout the day or over months in to provide a rough composite index of cortisol over time. For example, Ciccheti et al. (2001) collected four salivary cortisol samples every six months over the span of two years and van der Vegt et al. (2009) that collected four samples of salivary cortisol in one day. While both these methods were more stable than single point estimates, they still do not possess the same stability provided by hair samples. However, there are limitations in the use of hair as a measure of chronic cortisol for males due to the differential ability to collect hair samples across sex. Due to inadequate hair lengths from the back of the scalp for males where short hair or shaved scalps was fashionable at the time of testing, only 35% (23) of males had available cortisol data compared to 93% (78) of females. As the current study requires a length of 3 centimetres of hair taken from the back of the head to estimate a 3-month chronic cortisol level assuming a growth rate of 1 cm per month, there was a lack of male hair samples compared to females due to inadequate lengths of hair. Therefore, future analysis could examine different methods of obtaining samples for chronic cortisol levels such as fingernails or toenails. Another alternative is to impute the missing cortisol data by first using available cortisol data to produce a trend line. The slope and the individual variations from the original data could provide the information to compute a missing data algorithm for the imputation.

5.2 Conclusion

The effects of ACEs on biological stress response systems operate through molecular mechanisms which are largely responsible for the amplification of pro-inflammatory signals in immune cells causing physiological changes that drive the development of chronic diseases. The current study investigated the relationship between ACEs and biological stress systems such as the HPA axis and inflammation among young adults through chronic cortisol and inflammatory receptors.

From the analyses, ACEs were associated with IL-6Rα. However, ACEs were trending towards a significant association in inflammatory analytes including CRP, gp130, and IFN-γ but the pilot sample was small and underpowered, therefore the analyses was unable to properly detect associations with respect to the physiological measures. The findings also indicate that increased exposure to ACEs were associated with decreased cortisol levels. Contrary to what was hypothesized, lower cortisol levels associated with ACEs were similar to previous studies in which middle to older aged adults that reported past trauma but contrary to studies on children. Currently, the relationship between cortisol and the measure of inflammation by including pro-inflammatory receptors require further investigation but the negative correlation between cortisol and pro-inflammatory markers identified in the current study are consistent with the general effects of cortisol on inflammation. The lack of mediation of cortisol between ACEs and inflammatory markers suggests that dysregulation of cortisol may be a lifelong process that takes time to foster a chronic inflammatory environment where pro-inflammatory receptors are elevated.

Even though the sample size at the current time was underpowered for the proposed statistical analyses, the statistical trend found in regression models of the inflammatory analytes resembled other studies that examined cytokines. As ACEs were associated with IL-6Rα , while gp130 was marginally significant, the current study complements other studies that found associations between ACES and IL-6. In conclusion, the findings for cortisol, IL-6Rα, and the potential associations of other analytes point towards potential dysregulation of stress response systems that place ACEs exposed individuals in a trajectory that supports the development of chronic inflammation.

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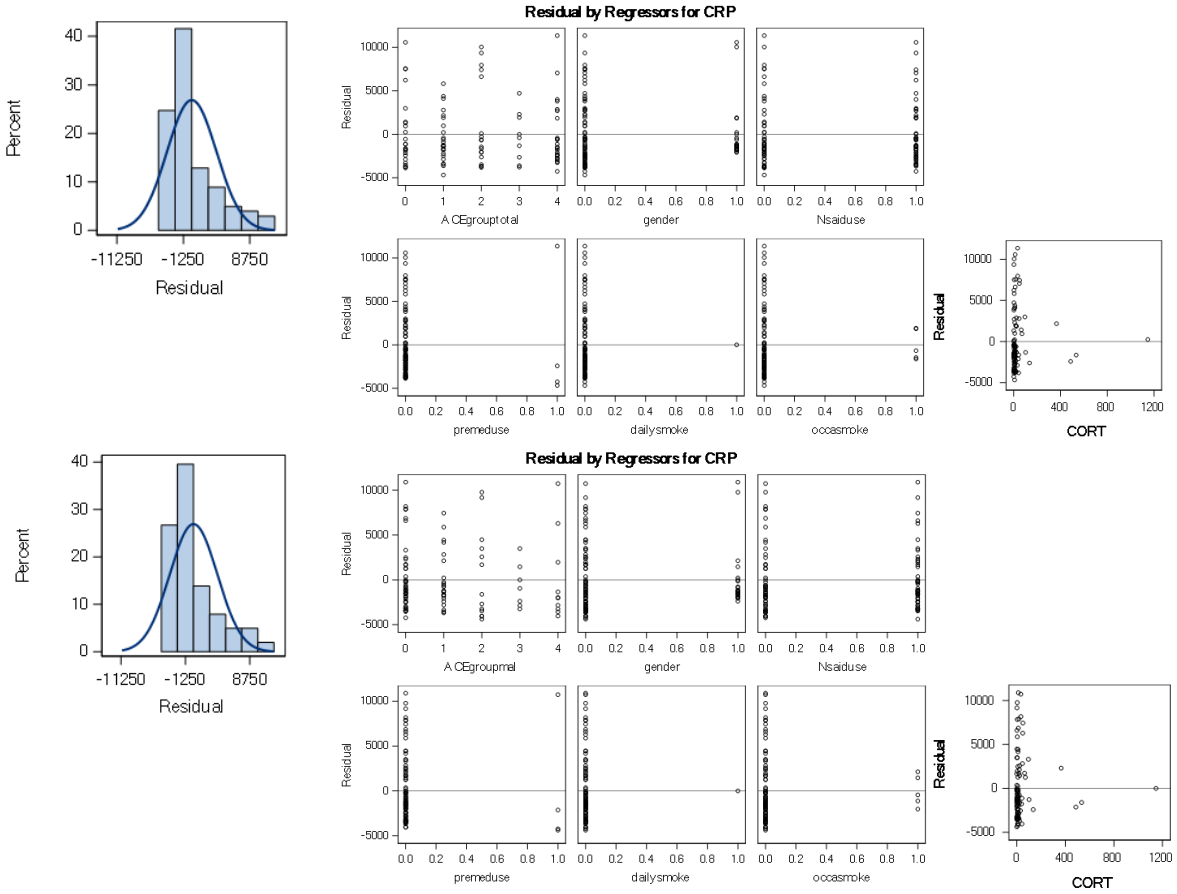
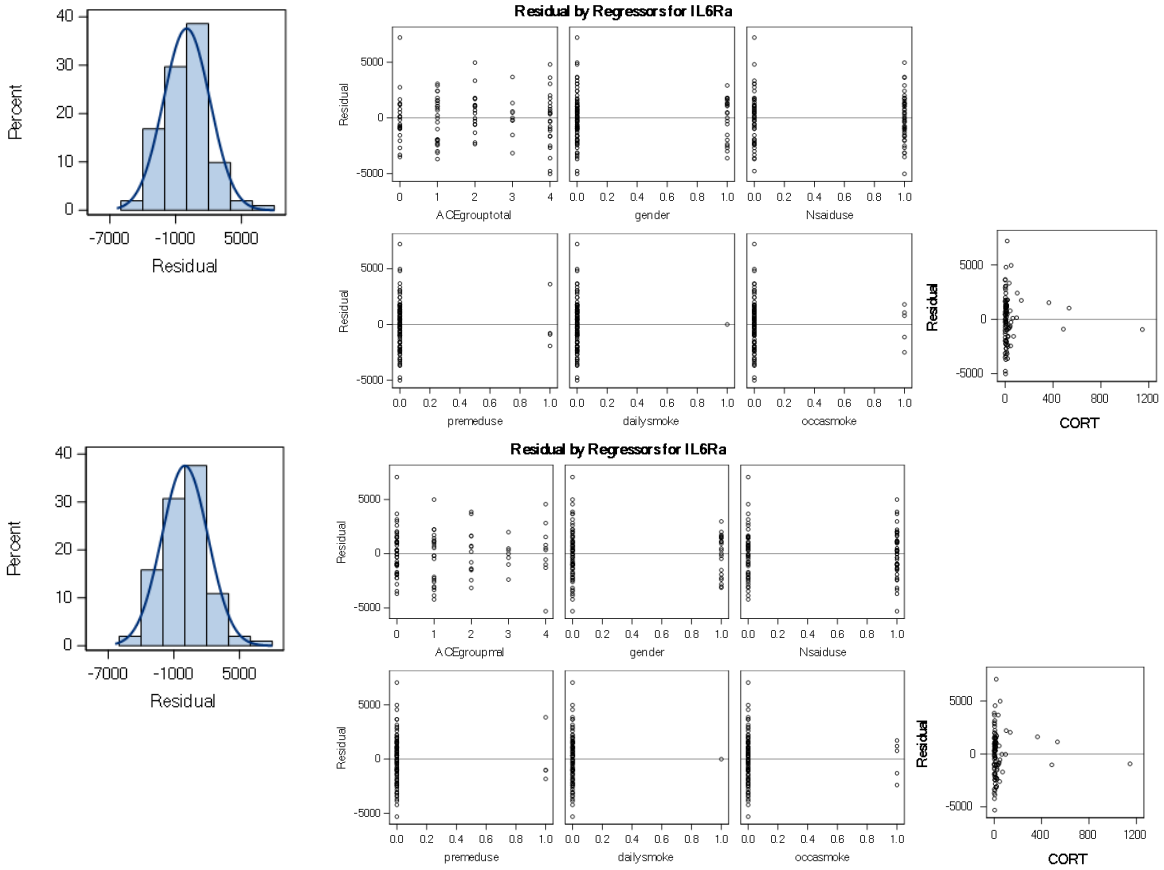
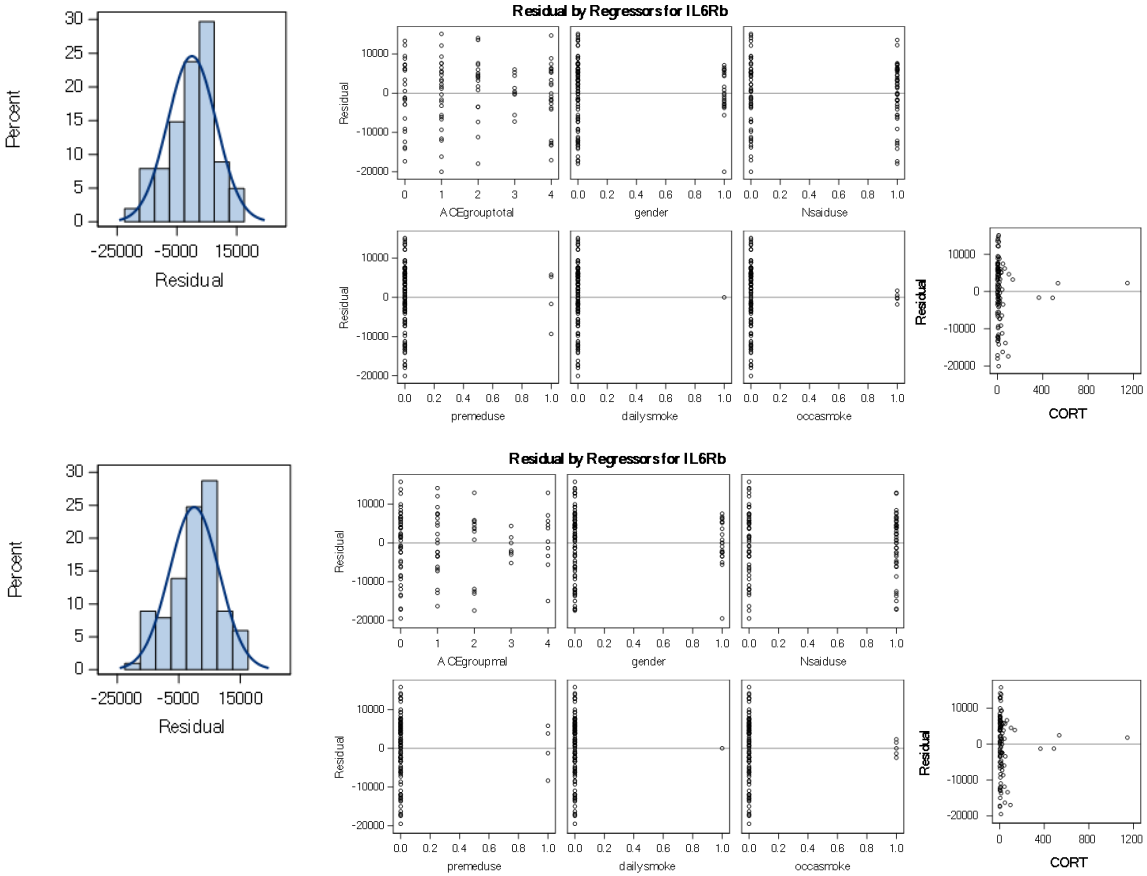


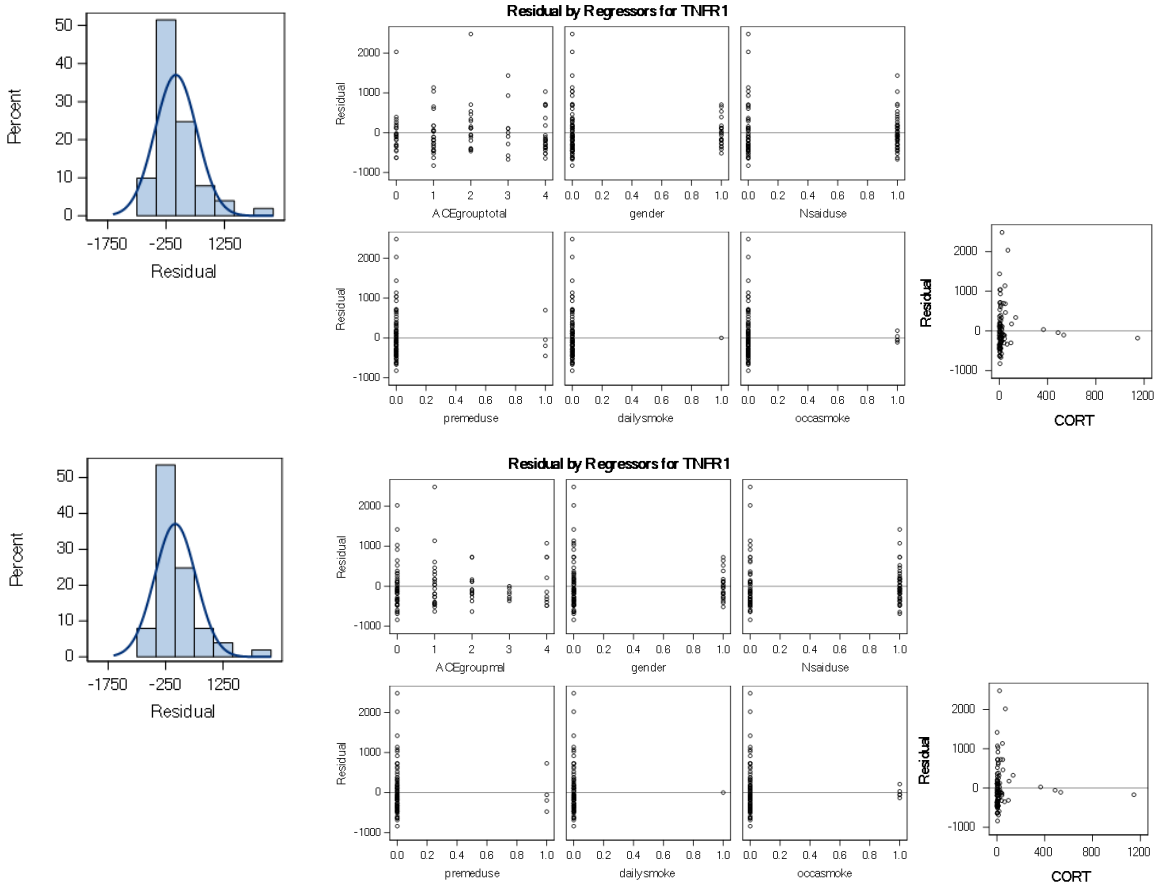
Supp. Figure 7.1 Histogram of residuals and residuals plots for the CRP



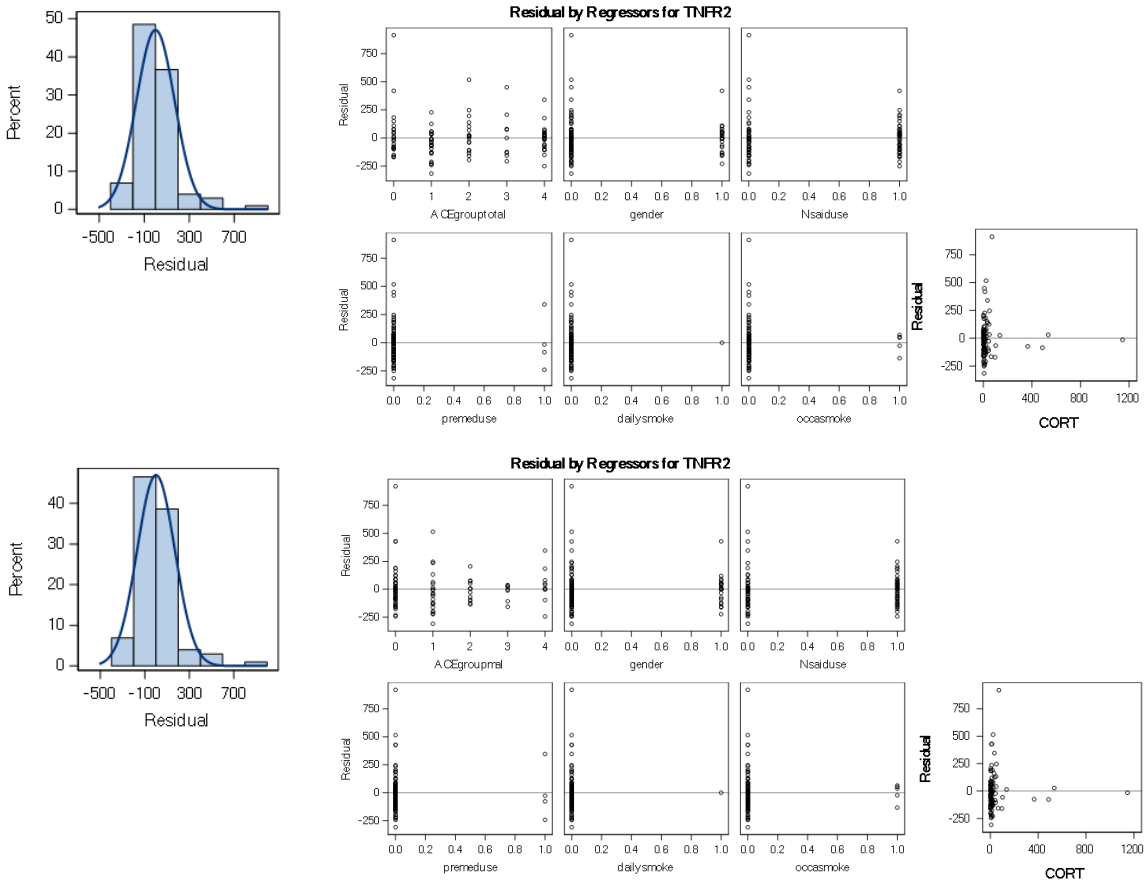
Supp. Figure 7.2 Histogram of residuals and residuals plots for the IL-6Rα



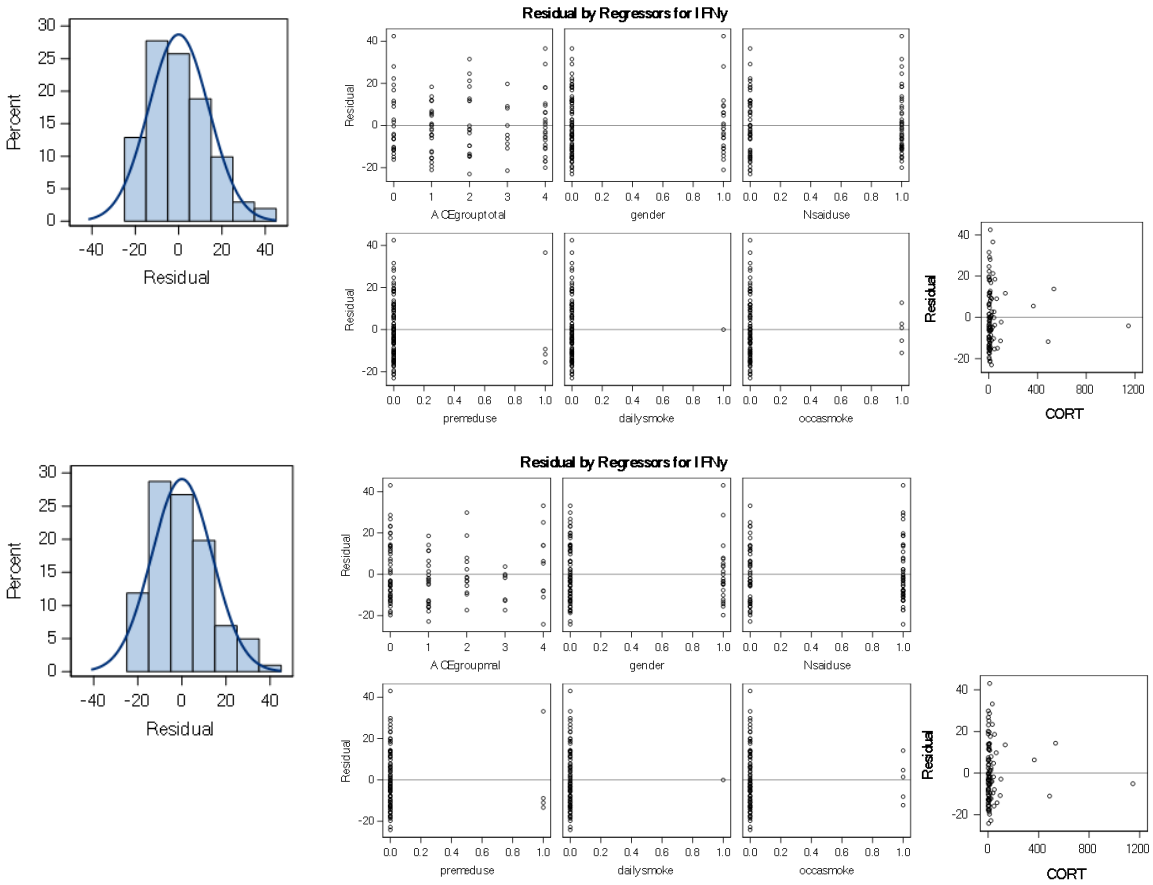
Supp. Figure 7.3 Histogram of residuals and residuals plots for the gp130



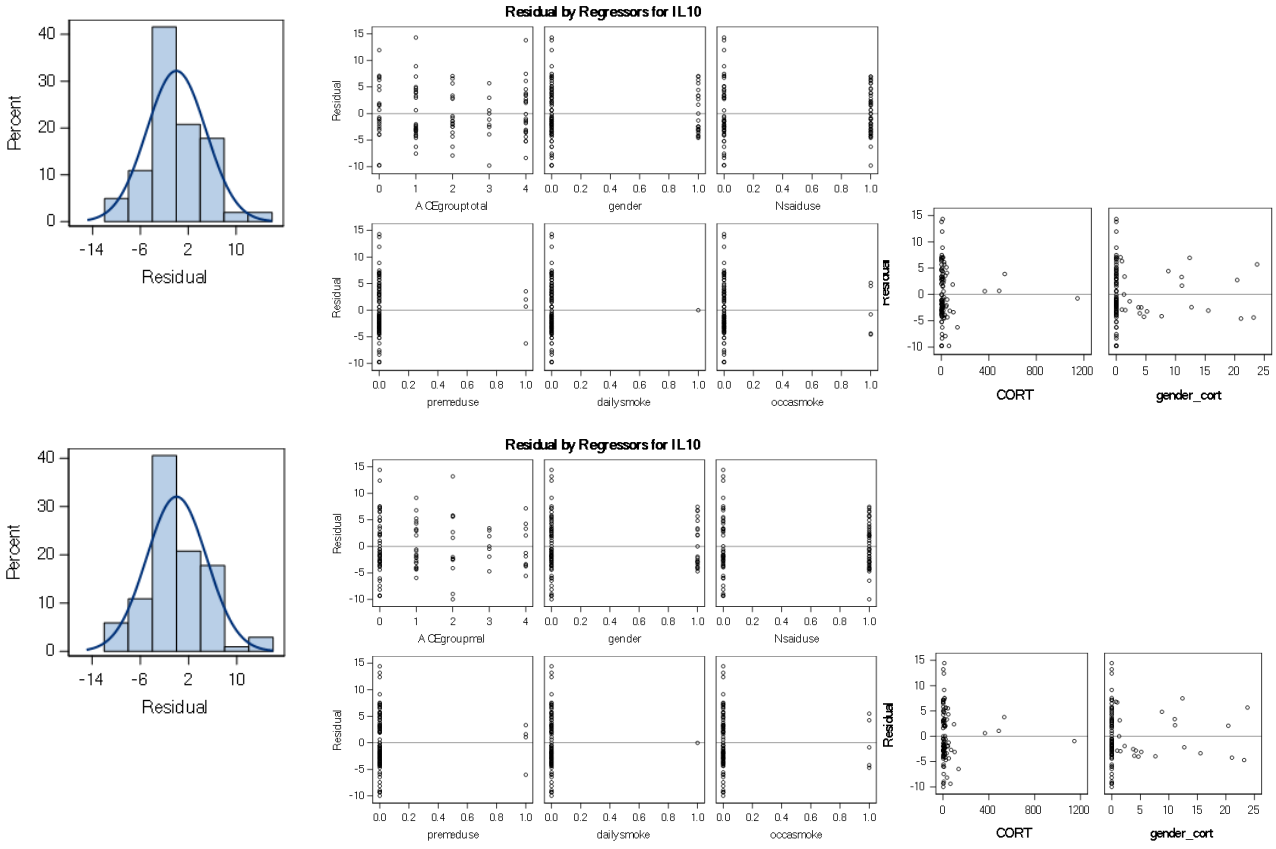
Supp. Figure 7.4 .Histogram of residuals and residuals plots for the sTNFr1



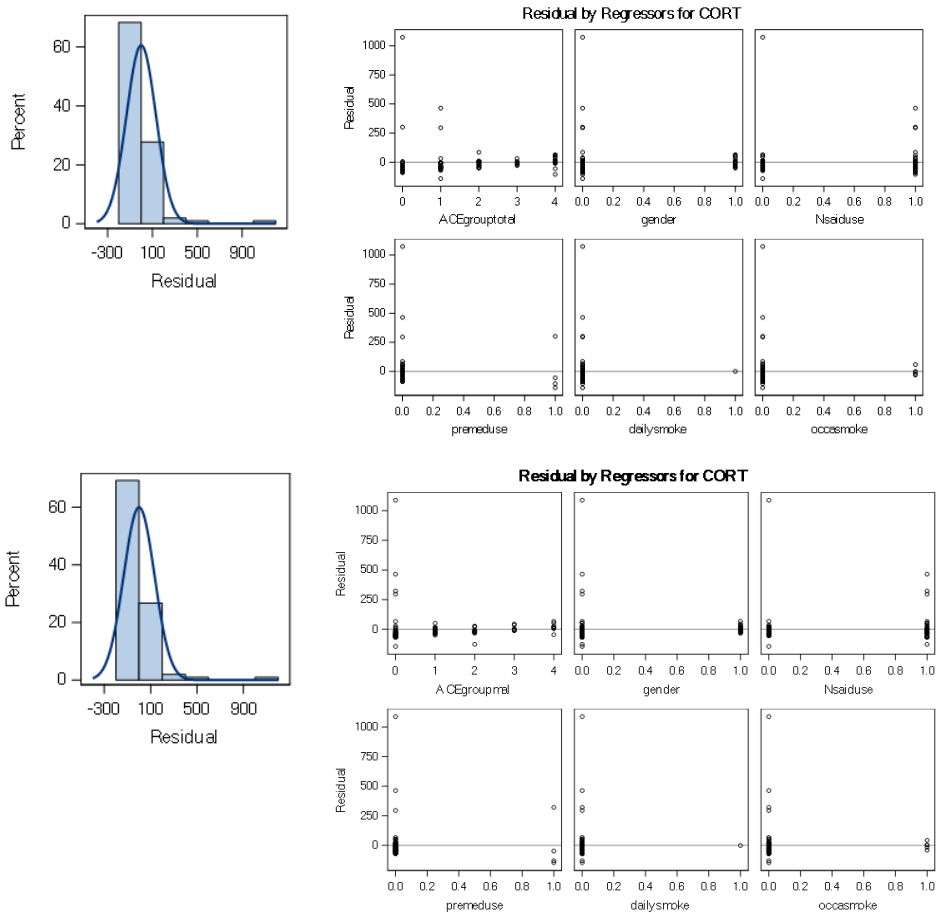
Supp. Figure 7.5 Histogram of residuals and residuals plots for the sTNFr2



Supp. Figure 7.6 Histogram of residuals and residuals plots for the IFN-γ



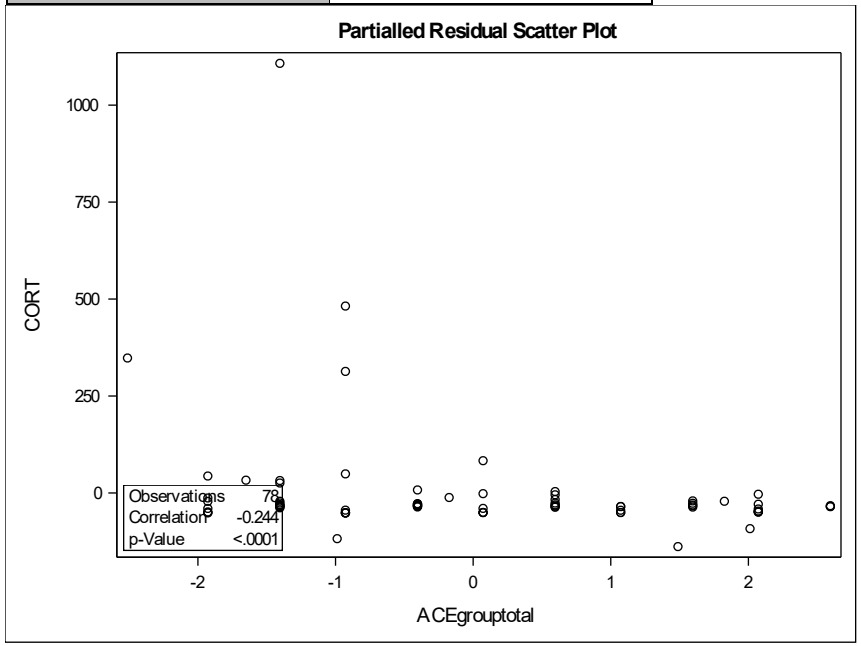
Supp. Figure 7.7 Histogram of residuals and residuals plots for the IL-10



Supp. Figure 7.8 Histogram of residuals and residuals plots for the cortisol

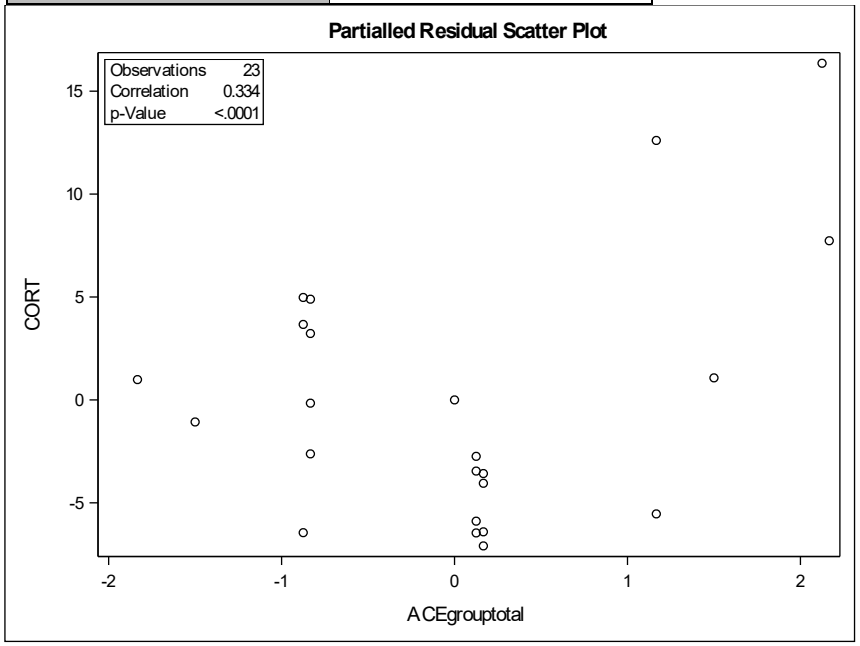
4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	CORT
1 Variables:	ACEgrouptotal

Pearson Partial Correlation Coefficients, N = 78	
	ACEgrouptotal
CORT **CORT**	-0.24409



4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	CORT
1 Variables:	ACEgrouptotal

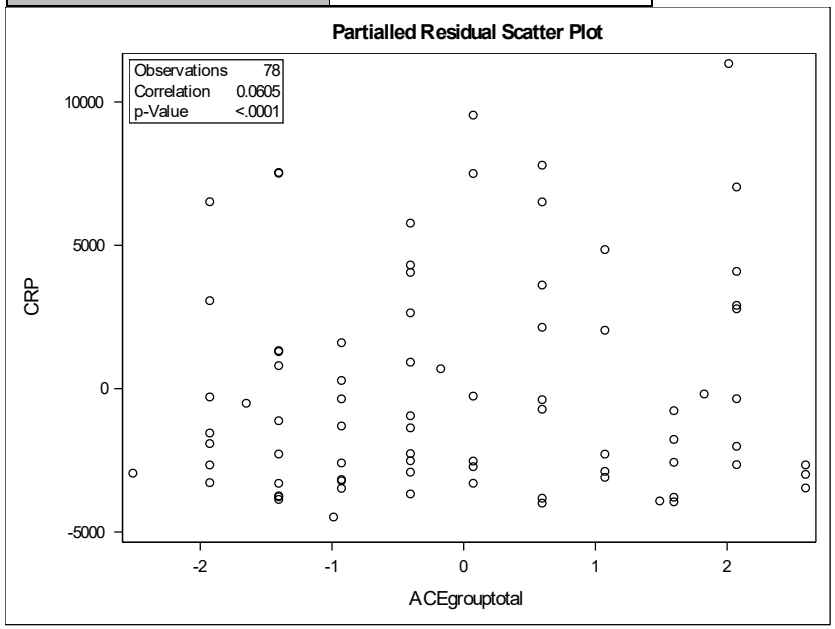
Pearson Partial Correlation Coefficients, N = 23	
	ACEgrouptotal
CORT **CORT**	0.33400



Supp. Figure 7.9 **Partial correlations of Total ACEs to cortisol by sex**

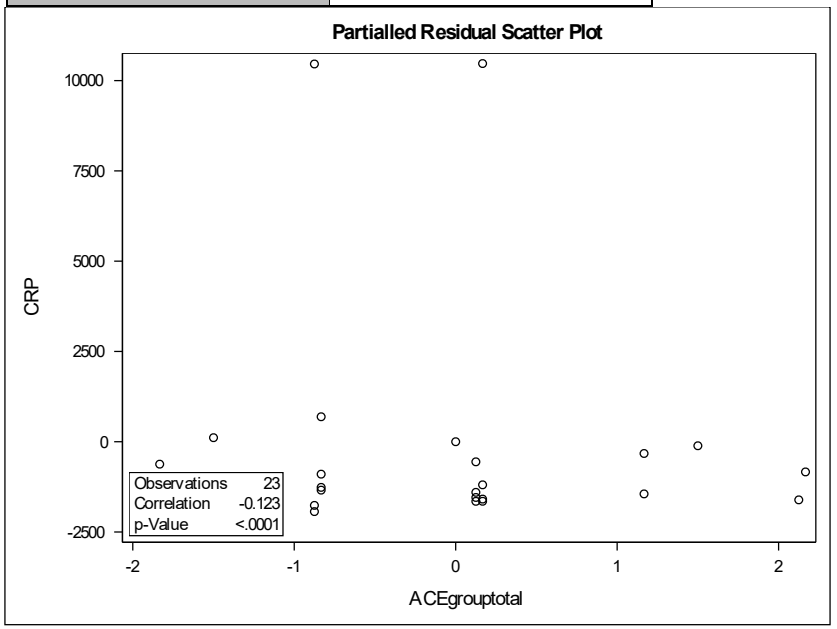
4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	CRP
1 Variables:	ACEgrouptotal

Pearson Partial Correlation Coefficients, N = 78	
	ACEgrouptotal
CRP CRP	0.06054



4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	CRP
1 Variables:	ACEgrouptotal

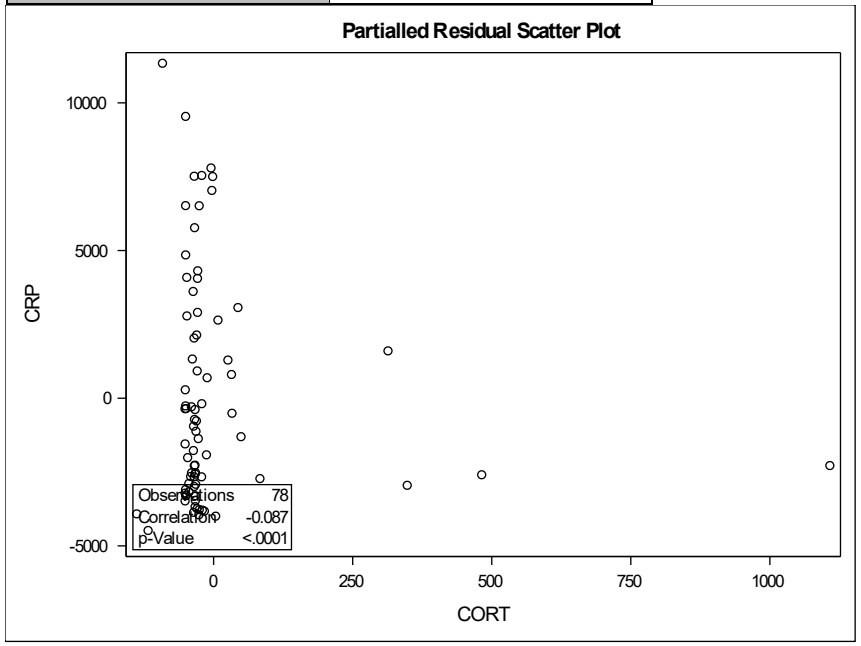
Pearson Partial Correlation Coefficients, N = 23	
	ACEgrouptotal
CRP CRP	-0.12253



Supp. Figure 7.10 **Partial correlations of Total ACEs to CRP by sex**

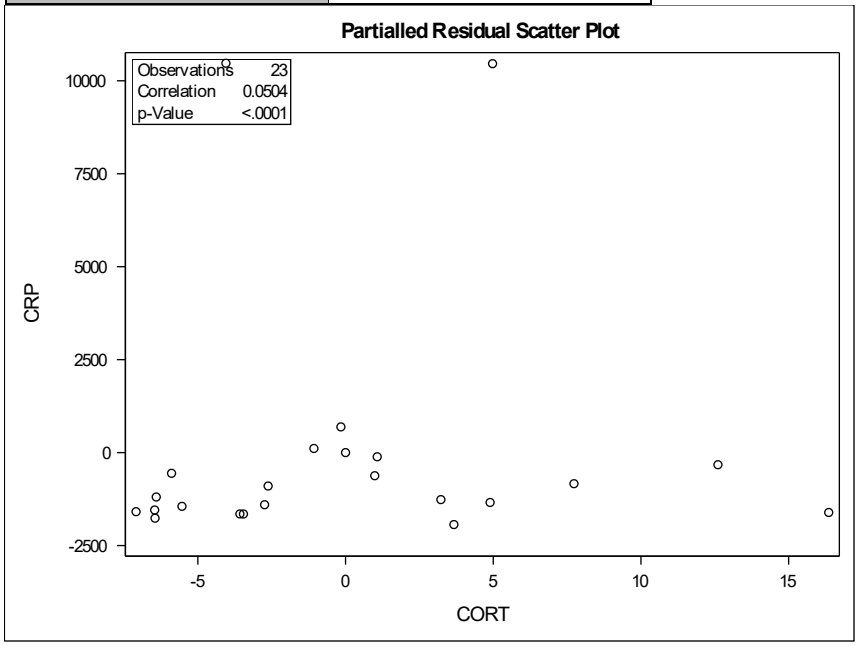
4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	CRP
1 Variables:	CORT

Pearson Partial Correlation Coefficients, N = 78	
	CORT
CRP CRP	-0.08672



4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	CRP
1 Variables:	CORT

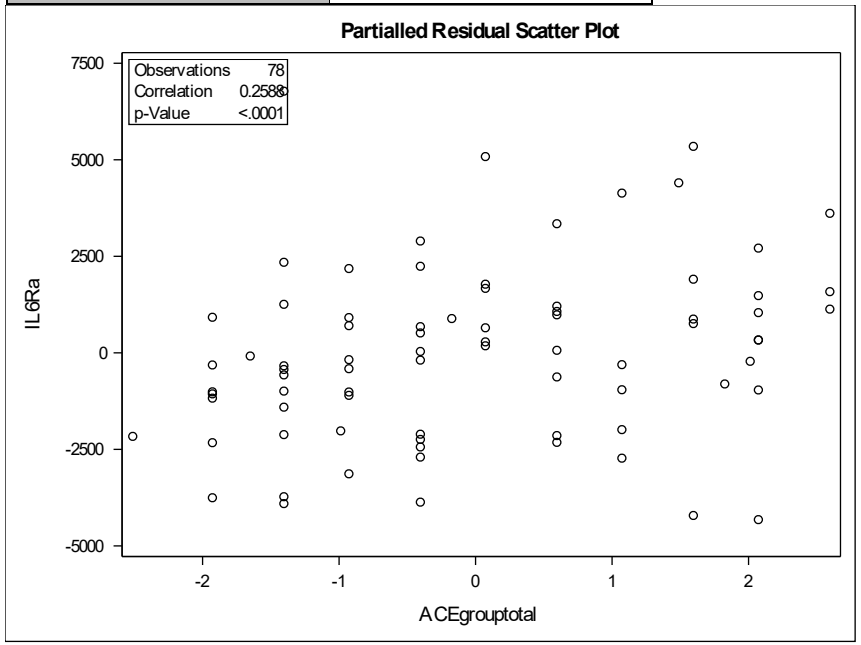
Pearson Partial Correlation Coefficients, N = 23	
	CORT
CRP CRP	0.05045



Supp. Figure 7.11 **Partial correlations of Cortisol to CRP by sex**

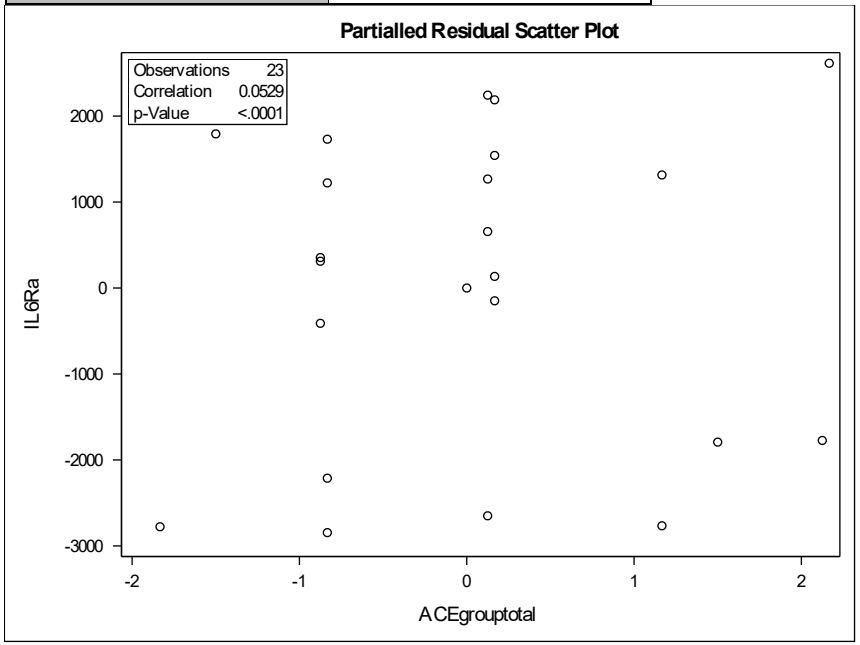
4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	IL6Ra
1 Variables:	ACEgrouptotal

Pearson Partial Correlation Coefficients, N = 78	
	ACEgrouptotal
IL6Ra IL6Ra	0.25877



4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	IL6Ra
1 Variables:	ACEgrouptotal

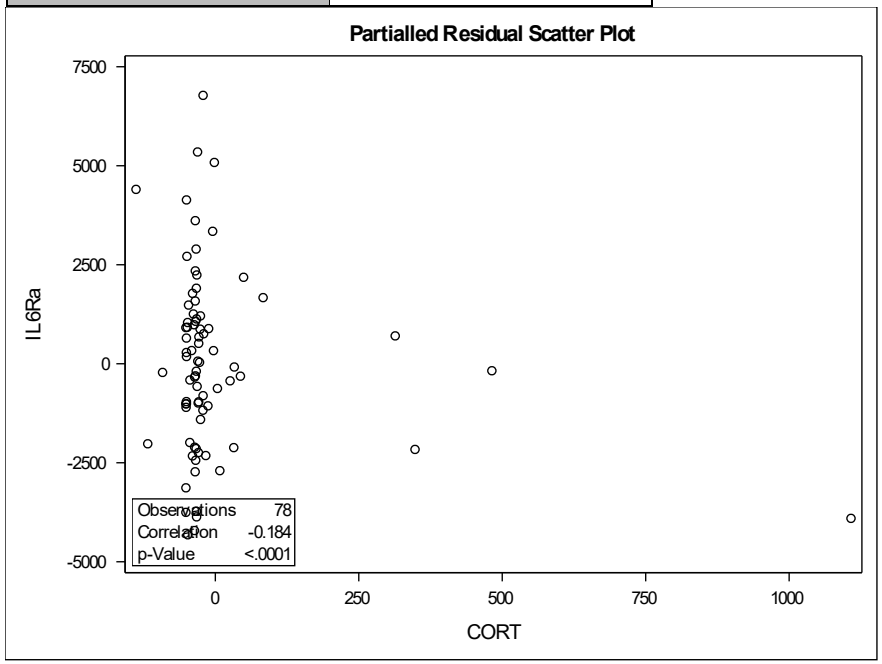
Pearson Partial Correlation Coefficients, N = 23	
	ACEgrouptotal
IL6Ra IL6Ra	0.05290



Supp. Figure 7.12 Partial correlations of Total ACEs to IL-6Rα by sex

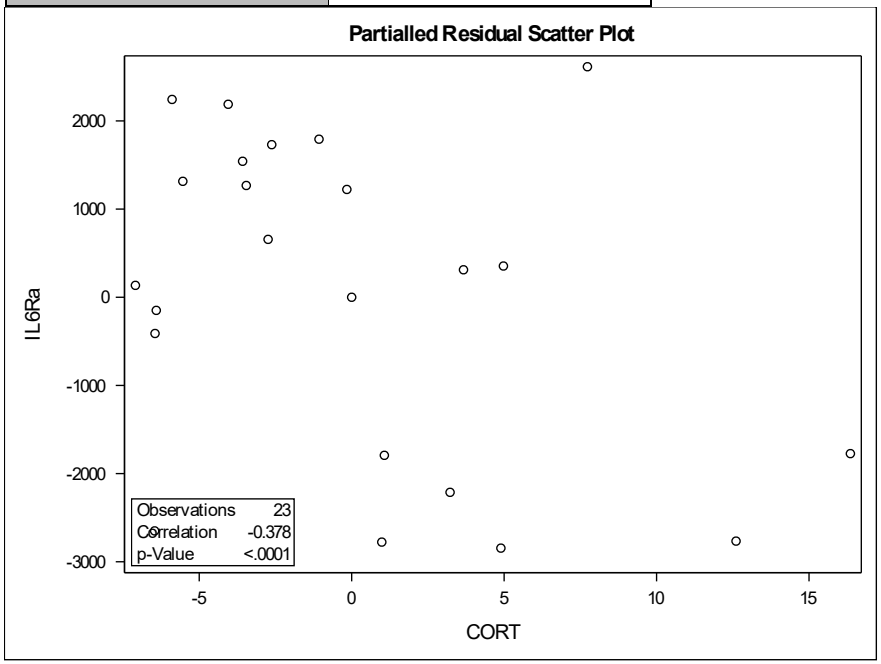
4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Varables:	IL6Ra
1 Variables:	CORT

Pearson Partial Correlation Coefficients, N = 78	
	CORT
IL6Ra IL6Ra	-0.18431



4 Partial Variables:	Nsaiduse premeduse dailysmoke occamoke
1 With Variables:	IL6Ra
1 Variables:	CORT

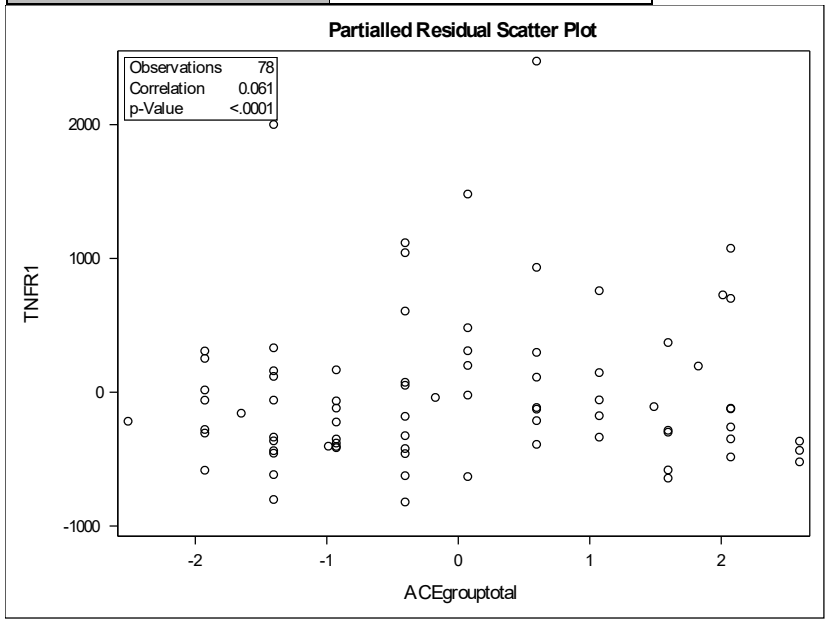
Pearson Partial Correlation Coefficients, N = 23	
	CORT
IL6Ra IL6Ra	-0.37818



Supp. Figure 7.13 Partial correlations of Cortisol to IL-6Rα by sex

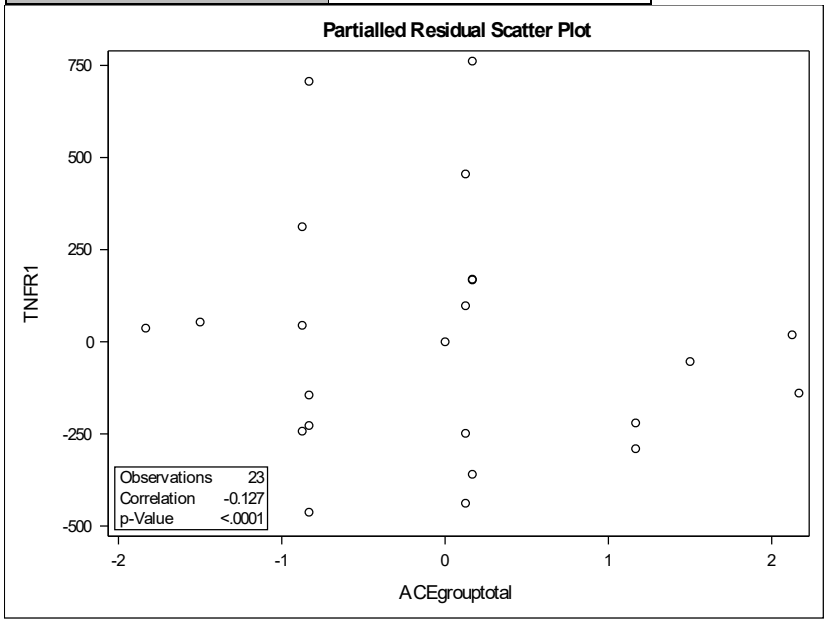
4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	TNFR1
1 Variables:	ACEgrouptotal

Pearson Partial Correlation Coefficients, N = 78	
	ACEgrouptotal
TNFR1 **TNFR1**	0.06095



4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 Wit Variables:	TNFR1
1 Variables:	ACEgrouptotal

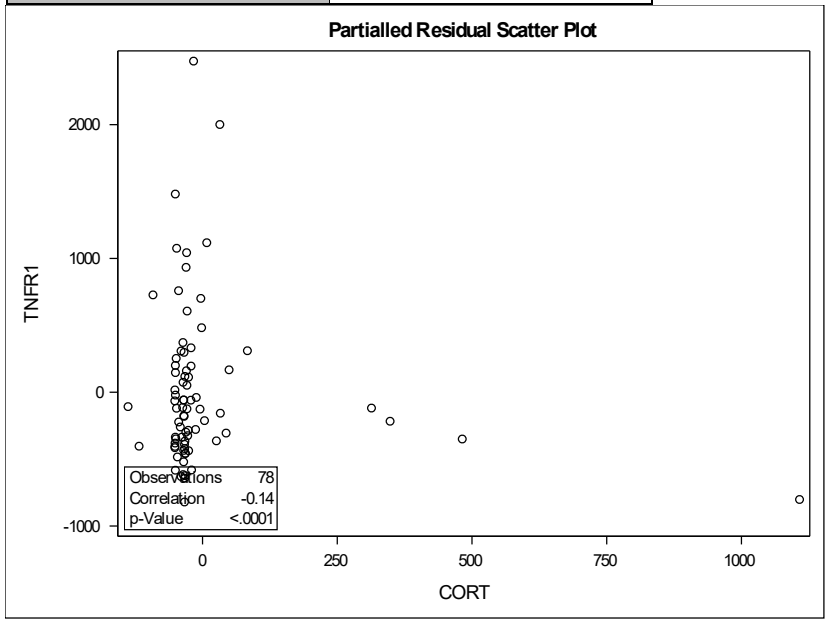
Pearson Partial Correlation Coefficients, N = 23	
	ACEgrouptotal
TNFR1 **TNFR1**	-0.12706



Supp. Figure 7.14 Partial correlations of Total ACEs to sTNFr1 by sex

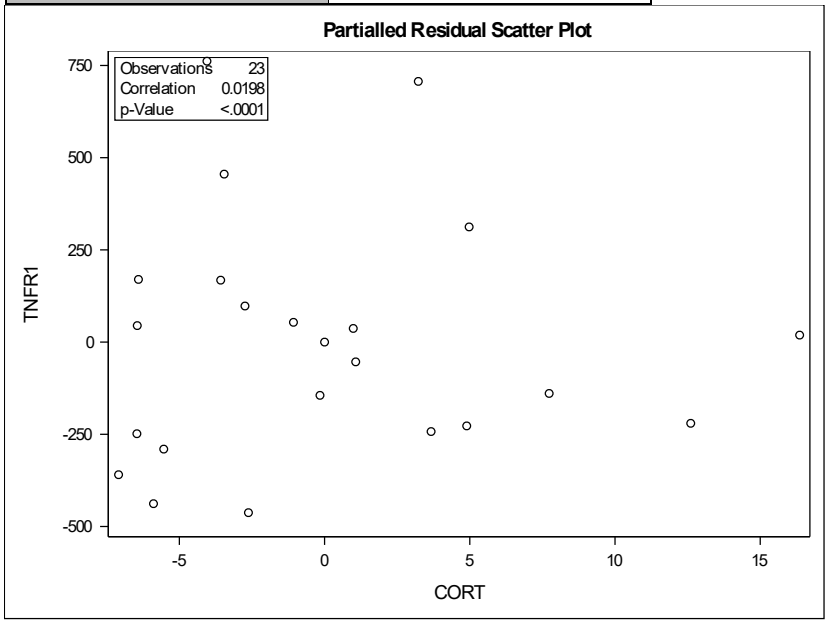
4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	TNFR1
1 Variables:	CORT

Pearson Partial Correlation Coefficients, N = 78	
	CORT
TNFR1 **TNFR1**	-0.13992



4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	TNFR1
1 Variables:	CORT

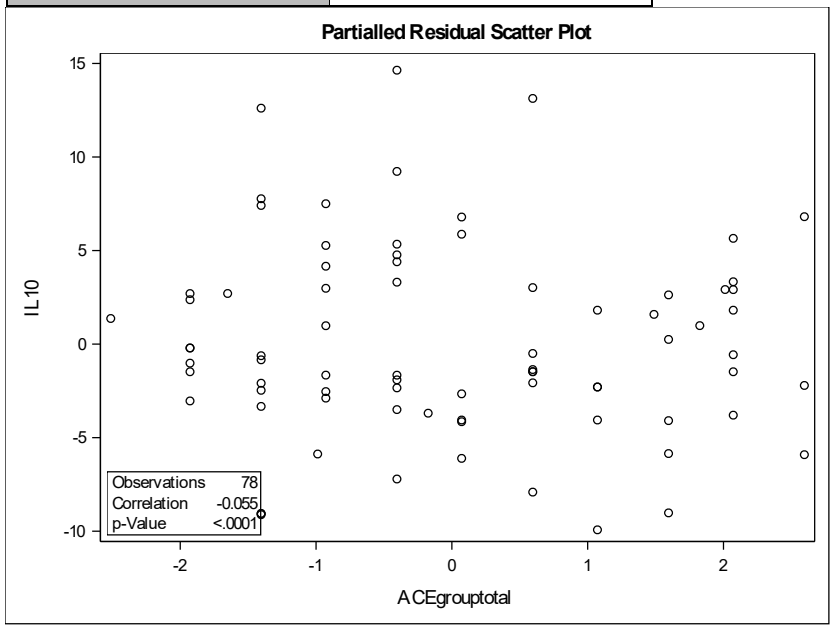
Pearson Partial Correlation Coefficients, N = 23	
	CORT
TNFR1 **TNFR1**	0.01977



Supp. Figure 7.15 **Partial correlations of Cortisol to sTNFr1 by sex**

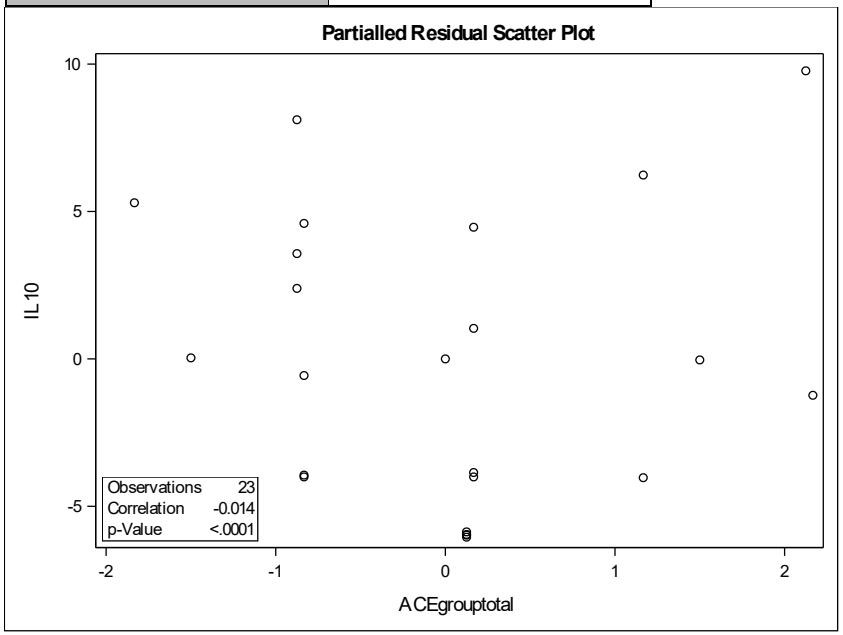
4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	IL10
1 Variables:	ACEgrouptotal

Pearson Partial Correlation Coefficients, N = 78	
	ACEgrouptotal
IL10 IL10	-0.05539



4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	IL10
1 Variables:	ACEgrouptotal

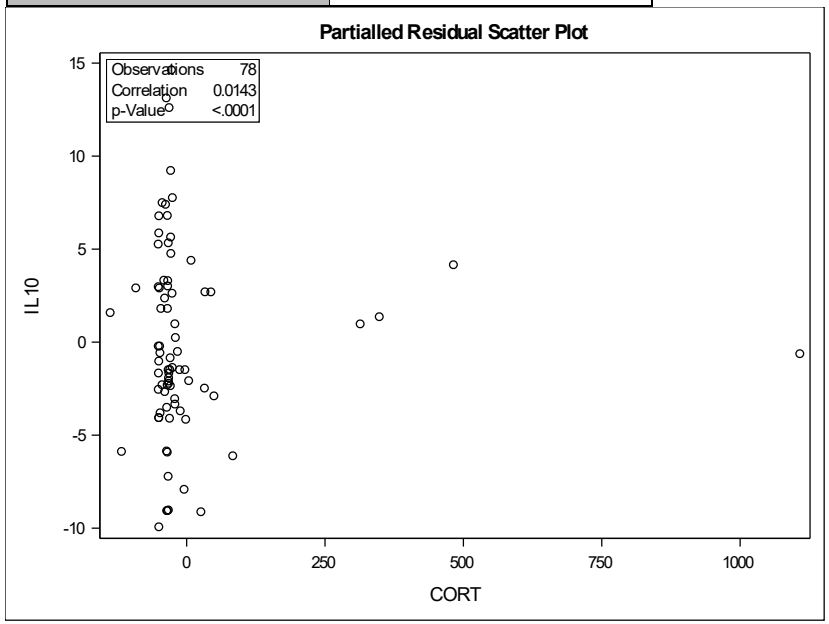
Pearson Partial Correlation Coefficients, N = 23	
	ACEgrouptotal
IL10 IL10	-0.01394



Supp. Figure 7.16 **Partial correlations of Total ACEs to IL-10 by sex**

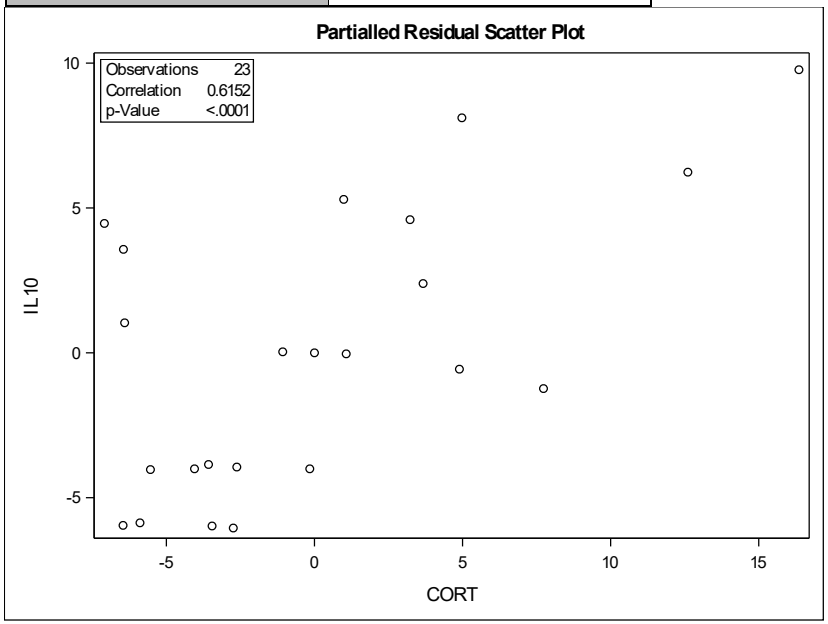
4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	IL10
1 Variables:	CORT

Pearson Partial Correlation Coefficients, N = 78	
	CORT
IL10 **IL10**	0.01433



4 Partial Variables:	Nsaiduse premeduse dailysmoke occasmoke
1 With Variables:	IL10
1 Variables:	CORT

Pearson Partial Correlation Coefficients, N = 23	
	CORT
IL10 **IL10**	0.61516



Supp. Figure 7.17 **Partial correlations of Cortisol to IL-10 by sex**